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<div style="text-align: center;"> <p style="text-align: right;">(I)</p> </div>			
(57) Abstract			
<p>Compounds of formula (I), wherein R¹ is an aryl, arylC₁₋₆alkyl, heteroaryl or heteroarylC₁₋₆alkyl group; R² is hydrogen, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₆cycloalkyl, heteroaryl, heterocyclyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl, heterocyclylC₁₋₆alkyl or C₃₋₆cycloalkylC₁₋₆alkyl; R³ is C₁₋₆alkyl, C₂₋₆alkenyl, aryl, C₁₋₆alkyl, heteroarylC₁₋₆alkyl or the side-chain of a naturally occurring amino acid; R⁴ is hydrogen, C₁₋₆alkyl, C₃₋₆cycloalkyl, C₄₋₆cycloalkenyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl or heterocyclylC₁₋₆alkyl; R⁵ is hydrogen or C₁₋₆alkyl; or R⁴ and R⁵ together with the nitrogen atom to which they are joined form a heterocyclic ring; wherein any group or ring, in R¹⁻⁵, is optionally substituted; or pharmaceutically acceptable salts or <i>in vivo</i> hydrolysable esters thereof, are described as inhibitors of the production of Tumour Necrosis Factor and/or one or more matrix metalloproteinase enzymes. Compositions containing them and their preparation are also described.</p>			

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THIO DERIVATIVES OF HYDROXAMIC ACIDS

This invention relates to thio compounds and in particular to thio compounds wherein a thio substituent is located adjacent to a hydroxycarbamate group. This invention
5 further relates to processes for preparing such thio compounds, to pharmaceutical compositions containing them and to their use in methods of therapeutic treatment.

The compounds of this invention are inhibitors of the production of TNF (Tumour Necrosis Factor) which is believed to be formed by the cleavage of a pro-form, or larger precursor, by the enzyme pro-TNF Convertase. Applicants believe that the compounds of the
10 present invention inhibit TNF production by mechanisms which include inhibition of pro-TNF Convertase. The term 'TNF' is used herein to refer to Tumour Necrosis Factor in general but, in particular, to TNF α .

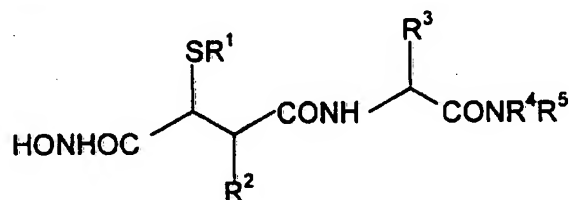
The compounds of this invention will be useful in the treatment of disease or medical conditions in which excessive TNF production is known to give rise via a cascade of
15 processes to a variety of physiological sequelae including the production of physiologically-active eicosanoids such as the prostaglandins and leukotrienes, the stimulation of the release of proteolytic enzymes such as collagenase, the activation of osteoclast activity leading to the resorption of calcium, the stimulation of the release of proteoglycans from, for example, cartilage, the stimulation of cell proliferations and to angiogenesis. It is also known that, in
20 certain cellular systems, TNF production precedes and mediates the production of other cytokines such as interleukin-1 (IL-1) and interleukin-2 (IL-2) which are also believed to contribute to the pathology of disease states such as inflammatory and allergic diseases and cytokine-induced toxicity. Excessive TNF production has also been implicated in mediating or exacerbating the development of various inflammatory and allergic diseases such as
25 inflammation of the joints (especially rheumatoid arthritis, osteoarthritis and gout), inflammation of the gastrointestinal tract (especially inflammatory bowel disease, ulcerative colitis and gastritis), skin disease (especially psoriasis, eczema and dermatitis) and respiratory disease (especially asthma, bronchitis and allergic rhinitis), and in the production and development of various cardiovascular disorders such as myocardial infarction, angina and
30 peripheral vascular disease. Excessive TNF production has also been implicated in mediating complications of bacterial, fungal and/or viral infections such as endotoxic shock, septic

shock and toxic shock syndrome. Excessive TNF production has also been implicated in mediating or exacerbating the development of adult respiratory distress syndrome, diseases involving cartilage or muscle resorption, Paget's disease and osteoporosis, pulmonary fibrosis, cirrhosis, renal fibrosis, the cachexia found in certain chronic diseases such as
 5 malignant disease and acquired immune deficiency syndrome (AIDS), tumour invasiveness and tumour metastasis and multiple sclerosis.

The compounds of the invention may also be inhibitors of one or more matrix metalloproteinases such as collagenases, stromelysins and gelatinases. Thus they may also be
 10 of use in the therapeutic treatment of disease conditions mediated by such enzymes for example arthritis (rheumatoid and osteoarthritis), osteoporosis and tumour metastasis.

The present invention provides novel thio compounds which have activity as inhibitors of TNF production and/or are inhibitors of one or more matrix metalloproteinase enzymes.

Accordingly the present invention provides a compound of the formula (I):



15

(I)

wherein:

- R¹ is is aryl, arylC₁₋₆alkyl, heteroaryl or heteroarylC₁₋₆alkyl;
 20 R² is is hydrogen, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₆cycloalkyl, aryl, heteroaryl, heterocyclyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl, heterocyclylC₁₋₆alkyl or C₃₋₆cycloalkylC₁₋₆alkyl;
 R³ is C₁₋₆alkyl, C₂₋₆alkenyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl or the side-chain of a naturally occurring amino acid;
 25 R⁴ is hydrogen, C₁₋₆alkyl, C₃₋₆cycloalkyl, C₄₋₆cycloalkenyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl or heterocyclylC₁₋₆alkyl;
 R⁵ is hydrogen or C₁₋₆alkyl; or R⁴ or R⁵ together with the nitrogen atom to which they are joined form a heterocyclic ring;

wherein any group or ring, in R^1 - R^5 , is optionally substituted;

or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof.

"Aryl in the terms "aryl" and "aryl C_{1-6} alkyl" typically means phenyl or naphthyl, preferably phenyl. "Heteroaryl" in the terms "heteroaryl" and "heteroaryl C_{1-6} alkyl" means an aromatic mono- or bicyclic 5-10 membered ring with up to five ring heteroatoms selected from nitrogen, oxygen and sulphur. Examples of 'heteroaryl' include thienyl, pyrrolyl, furanyl, imidazolyl, thiazolyl, pyrimidinyl, pyridinyl, indolyl, benzimidazolyl, benzthiazolyl, quinolinyl and isoquinolinyl. "Heterocyclyl" in the terms "heterocyclyl" and heterocyclyl- C_{1-6} alkyl means a non-aromatic mono- or bicyclic 5-10 membered ring with up to five ring hetero atoms selected from nitrogen, oxygen and sulphur. Examples of 'heterocyclyl' include pyrrolidinyl, morpholinyl, piperidinyl, dihydropyridinyl and dihydropyrimidinyl.

Any group or ring in R^1 - R^5 may be optionally substituted, for example by up to three substituents which may be the same or different. Typical substituents include: hydroxy, C_{1-6} alkoxy for example methoxy, mercapto, C_{1-6} alkylthio for example methylthio, amino, C_{1-6} alkylamino for example methylamino, di- $(C_{1-6}$ alkyl)amino for example dimethylamino, carboxy, carbamoyl, C_{1-6} alkylcarbamoyl for example methylcarbamoyl, di- C_{1-6} alkylcarbamoyl for example dimethylcarbamoyl, C_{1-6} alkylsulphonyl for example methylsulphonyl, arylsulphonyl for example phenylsulphonyl, C_{1-6} alkylaminosulphonyl for example methylaminosulphonyl, di- $(C_{1-6}$ alkyl)aminosulphonyl for example dimethylamino-sulphonyl, nitro, cyano, cyano C_{1-6} alkyl for example cyanomethyl, hydroxy C_{1-6} alkyl for example hydroxymethyl, amino C_{1-6} alkyl for example aminoethyl, C_{1-6} alkanoylamino for example acetamido, C_{1-6} alkoxycarbonylamino for example methoxycarbonylamino, C_{1-6} alkanoyl for example acetyl, C_{1-6} alkanoyloxy for example acetoxy, C_{1-6} alkyl for example methyl, ethyl, isopropyl or tert-butyl, halo for example fluoro, chloro or bromo, trifluoromethyl, aryl for example phenyl, aryl C_{1-6} alkyl for example benzyl, aryloxy for example phenoxy, aryl C_{1-6} alkoxy for example benzyloxy, heteroaryl, heteroaryl C_{1-6} alkyl, heterocyclyl and heterocyclyl C_{1-6} alkyl. The term "side chain of a naturally occurring amino acid" means the side chain X of an amino acid NH_2 -CHX-COOH. Suitable amino acids include alanine, arginine, aspartic acid, cysteine, asparagine, glutamine, histidine, homoserine, isoleucine, leucine, lysine, methionine, norleucine, norvaline, ornithine, serine, threonine, tryptophan, tyrosine and valine.

The compounds of the present invention possess a number of chiral centres, at $-\text{CH}(\text{SR}^1)-$, at $-\text{CHR}^3-$, at $-\text{CHR}^2-$ (when R^2 is not hydrogen) and possibly in the variables R^1-R^5 . The present invention covers all diastereoisomers and mixtures thereof that inhibit pro-TNF Convertase and/or inhibit matrix metalloproteinase enzymes.

5 In one aspect R^1 is an optionally substituted aryl group. Suitably R^1 is an optionally substituted phenyl group. In another aspect R^1 is optionally substituted aryl C_{1-6} alkyl. In yet a further aspect R^1 is optionally substituted heteroaryl or heteroaryl- C_{1-6} alkyl.

Favourably R^1 is phenyl, phenyl C_{1-6} alkyl, naphthyl C_{1-6} alkyl, heteroaryl or
 10 heteroaryl C_{1-6} alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C_{1-6} alkylcarbonyl for example acetyl, C_{1-6} alkylsulphonyl for example methylsulphonyl, trifluoromethyl, cyano, C_{1-6} alkyl for example methyl, isopropyl or tert-butyl, C_{1-6} alkoxy for example methoxy, cyano C_{1-6} alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on a phenyl ring are linked to
 15 form a methylenedioxy ($-\text{OCH}_2\text{O}-$) group.

In another aspect R^1 is phenyl, phenyl C_{1-6} alkyl, naphthyl C_{1-6} alkyl, heteroaryl or heteroaryl C_{1-6} alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C_{1-6} alkylcarbonyl for example acetyl, C_{1-6} alkylsulphonyl for example methylsulphonyl, trifluoromethyl, C_{1-6} alkyl for
 20 example methyl, isopropyl or tert-butyl, C_{1-6} alkoxy for example methoxy, cyano C_{1-6} alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on a phenyl ring are linked to form a methylenedioxy ($-\text{OCH}_2\text{O}-$) group.

Further examples within the meaning of R^1 as an optionally substituted aryl group include those wherein two adjacent carbon atoms on a phenyl ring are linked by $-(\text{CH}_2)_m-$
 25 wherein m is 3 or 4, by $-\text{NR}^a-\text{CO}-(\text{CH}_2)_n-$ wherein R^a is hydrogen or C_{1-6} alkyl and n is 1 or 2, by $-\text{NR}^a-\text{COCH}=\text{CH}-$, $-\text{CO}-\text{NR}^a-(\text{CH}_2)_n-$ or by $-\text{CONR}^a-\text{CH}=\text{CH}-$.

Preferably R^1 is phenyl, 4-fluorophenyl, 4-trifluoromethylphenyl, 3,5-difluorophenyl, 4-acetylphenyl, 4-cyanophenyl, 4-methylsulphonylphenyl, 4-(1-cyano-1-methylethyl)phenyl, 3,4-dimethoxyphenyl, 3,5-dichlorophenyl or 3,5-di-trifluoromethyl-
 30 phenyl. Preferably also R^1 is 3-(1-cyano-1-methylethyl)phenyl, naphth-1-yl, 3-hydroxynaphth-7-yl or 2-chloro-4-fluorophenyl.

In a particular aspect R¹ is phenyl, 4-fluorophenyl, 4-trifluoromethylphenyl, 3,5-difluoro-phenyl, 4-acetylphenyl, 4-methylsulphonylphenyl, 4-(1-cyano-1-methylethyl)phenyl, 3,4-dimethoxyphenyl or 3,5-dichlorophenyl.

In particular R¹ may also be benzyl, phenethyl, phenylprop-1-yl, 1-methyl-
5 phenylmethyl (PhCHMe-), 1,1-dimethylphenylmethyl (PhCMe₂-), thiazolyl, benzthiazolyl, 4-methoxybenzyl, indolyl, benzimidazolyl, indolylmethyl, pyrimidinyl, quinolinyl for example quinolin-2-yl, quinolin-6-yl or quinolin-7-yl, isoquinolinyl, pyridinyl or quinolinylmethyl for example quinolin-8-ylmethyl. Of these benzthiazolyl, quinolin-2-yl, quinolin-8-ylmethyl and benzyl are preferred.

10 In a particular aspect R¹ may be benzyl, phenethyl, phenylprop-1-yl, 1-methyl-phenylmethyl (PhCHMe-), 1,1-dimethylphenylmethyl (PhCMe₂-), thiazolyl, benzthiazolyl, 4-methoxybenzyl, indolyl, benzimidazolyl or indolylmethyl.

Particularly also R¹ may be 1-methyl-2-oxo-quinolin-6-yl, 1-methyl-2-oxodihydro-quinolinyl, 1-methyl-2-oxotetrahydroquinolinyl, 2-methyl-1-oxodihydroisoquinolinyl or 2-
15 methyl-1-oxotetrahydroisoquinolinyl; of these 1-methyl-2-oxotetrahydroquinolin-7-yl is preferred.

Particular groups for R² include C₁₋₈alkyl for example isopropyl, n-propyl, isobutyl, sec-butyl, n-butyl, tert-butyl, isopentyl, n-pentyl, hexyl, heptyl or octyl; C₁₋₈alkyl interrupted by an oxygen or sulphur atom for example methoxypropyl, ethoxyethyl, propoxymethyl, ethylthioethyl, methylthiopropyl; phenylC₁₋₆alkyl for example benzyl, phenethyl,
20 phenylpropyl or phenylbutyl; phenylC₁₋₆alkyl wherein the alkyl chain is interrupted by oxygen or sulphur for example benzyloxypropyl and benzyloxybutyl; C₃₋₈cycloalkyl for example cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl; or C₃₋₈cycloalkylC₁₋₆alkyl for example cyclopropylmethyl, cyclopropylethyl, cyclobutylmethyl, cyclopentylmethyl or
25 cyclohexylmethyl.

In a particular aspect R² may be C₁₋₈alkyl for example isopropyl, n-propyl, isobutyl, sec-butyl, n-butyl, tert-butyl, isopentyl, n-pentyl, hexyl, heptyl or octyl; C₁₋₈alkyl interrupted by an oxygen or sulphur atom for example methoxypropyl, ethoxyethyl, propoxymethyl, ethylthioethyl, methylthiopropyl; phenylC₁₋₆alkyl for example benzyl, phenethyl,
30 phenylpropyl or phenylbutyl; C₃₋₈cycloalkyl for example cyclopropyl, cyclobutyl, cyclopentyl

or cyclohexyl; or C_{3-6} cycloalkyl C_{1-6} alkyl for example cyclopropylmethyl, cyclopropylethyl, cyclobutylmethyl, cyclopentylmethyl or cyclohexylmethyl.

Preferably R^2 is isobutyl.

There is a chiral centre at $-CHR^2-$ (when R^2 is not hydrogen); it is preferred that this
5 centre has the configuration indicated in formula (II) hereinafter. For most values of R^2 this centre will have the S-stereochemistry under the Cahn-Prelog-Ingold sequence rules.

Particular groups for R^3 include C_{1-6} alkyl for example methyl, ethyl, isopropyl, n-propyl, n-butyl, isobutyl, sec-butyl, tert-butyl, isopentyl, n-pentyl or hexyl; C_{1-6} alkyl interrupted by an oxygen or sulphur atom for example methoxyethyl, methoxypropyl,
10 methylthioethyl or 1,1-dimethylmethylthiomethyl ($MeSCMe_2-$); or phenyl C_{1-6} alkyl for example benzyl or phenethyl.

Preferably R^3 is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl with tert-butyl being most preferred.

The chiral centre at $-CHR^3-$ preferably has the S-configuration indicated in formula
15 (II) hereinafter. For most of R^3 this centre will have the S-stereochemistry.

Particular groups for R^4 include C_{1-6} alkyl for example methyl, ethyl, n-propyl, isopropyl, tert-butyl or n-butyl; C_{1-6} alkyl interrupted by an oxygen or sulphur atom for example hydroxyethyl, methoxyethyl, methylthioethyl or ethoxyethyl; phenyl C_{1-6} alkyl for example benzyl, phenethyl or phenylpropyl; or C_{3-6} cycloalkyl C_{1-6} alkyl for example
20 cyclopropylmethyl, cyclobutylmethyl or cyclopentylmethyl.

Particularly also, R^4 may be C_{1-6} alkylamino C_{2-6} alkyl for example methylaminoethyl, di- C_{1-6} alkylamino C_{2-6} alkyl for example dimethylaminoethyl or heterocyclic C_{1-6} alkyl for example 2-morpholinoethyl, 2-piperidinoethyl, 2-piperazinoethyl or 2-(N-methyl)piperazinoethyl.

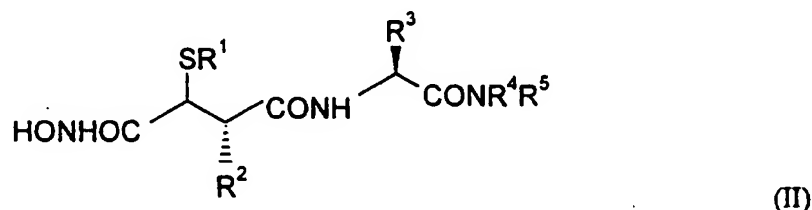
25 Preferably R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl or benzyl. In one aspect R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl or benzyl. Of these methyl is most preferred.

Particular groups for R^5 are hydrogen and C_{1-6} alkyl for example methyl or ethyl. Preferably R^5 is hydrogen.

In another aspect R^4 and R^5 together with the nitrogen atom to which they are joined form a heterocyclic ring, for example a 5 or 6 membered heterocyclic ring such as morpholino, piperidino, piperazino or N-methylpiperazino. Of these morpholino is preferred.

A particularly suitable class of compounds of the present invention is that of formula

5 (II):



wherein R^1 , R^2 , R^3 , R^4 and R^5 are as hereinbefore defined.

A preferred class of compounds of the formula (II) is that wherein R^1 is phenyl
 10 unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro; C_{1-6} alkylsulphonyl for example methylsulphonyl; trifluoromethyl; C_{1-6} alkyl for example methyl, isopropyl or tert-butyl; C_{1-6} alkoxy for example methoxy; cyano; C_{1-6} alkanoyl for example acetyl; cyano C_{1-6} alkyl for example 1-cyano-1-methylethyl; or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy ($-\text{OCH}_2\text{O}-$) group; R^2 is
 15 isobutyl; R^3 is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl, 2-morpholinoethyl or benzyl; and R^5 is hydrogen or methyl, or R^4 and R^5 together with the nitrogen atom to which they are joined form a morpholine ring.

In one aspect a preferred class of compounds of the formula (II) is that wherein R^1 is
 20 phenyl unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C_{1-6} alkylsulphonyl for example methylsulphonyl, trifluoromethyl, C_{1-6} alkyl for example methyl, isopropyl or tert-butyl, C_{1-6} alkoxy for example methoxy, cyano C_{1-6} alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy ($-\text{OCH}_2\text{O}-$) group; R^2 is isobutyl; R^3 is
 25 isobutyl, tert-butyl or benzyl; R^4 is methyl, ethyl, n-propyl, isobutyl, tert-butyl or benzyl; and R^5 is hydrogen.

A further preferred class of compounds of the formula (II) is that wherein R¹ is phenylC₁₋₆alkyl, heteroaryl or heteroarylC₁₋₆alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro, C₁₋₆alkylsulphonyl for example methylsulphonyl, trifluoromethyl, C₁₋₆alkyl for
 5 example methyl, isopropyl or tert-butyl, C₁₋₆alkoxy for example methoxy, cyano, C₁₋₆alkanoyl for example acetyl, cyanoC₁₋₆alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy (-OCH₂O-) group; R² is isobutyl; R³ is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R⁴ is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl, 2-morpholinoethyl or benzyl; and R⁵
 10 is hydrogen or methyl, or R⁴ and R⁵ together with the nitrogen atom to which they are joined form a morpholine ring.

In another aspect a class of compounds of the formula (II) is that wherein R¹ is phenylC₁₋₆alkyl, heteroaryl or heteroarylC₁₋₆alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen for example chloro or fluoro,
 15 C₁₋₆alkylsulphonyl for example methylsulphonyl, trifluoromethyl, C₁₋₆alkyl for example methyl, isopropyl or tert-butyl, C₁₋₆alkoxy for example methoxy, cyanoC₁₋₆alkyl for example 1-cyano-1-methylethyl, or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy (-OCH₂O-) group; R² is isobutyl; R³ is isobutyl, tert-butyl or benzyl; R⁴ is methyl, ethyl, n-propyl, isobutyl, tert-butyl or benzyl; and R⁵ is hydrogen.

20 In yet another aspect, a preferred class of compounds is that wherein R¹ is quinoliny, isoquinoliny, 1-methyl-2-oxodihydroquinoliny, 1-methyl-2-oxotetrahydroquinoliny, 2-methyl-1-oxodihydroisoquinoliny or 2-methyl-1-oxotetrahydroisoquinoliny; R² is isobutyl; R³ is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R⁴ is methyl, ethyl, n-propyl, isobutyl, tert-butyl,
 25 dimethylaminoethyl, 2-morpholinoethyl or benzyl; and R⁵ is hydrogen or methyl, or R⁴ and R⁵ together with the nitrogen atom to which they are joined form a morpholine ring.

Suitable pharmaceutically acceptable salts include acid addition salts such as hydrochloride, hydrobromide, citrate and maleate salts and salts formed with phosphoric and sulphuric acid. In another aspect suitable salts are base salts such as an alkali metal salt for
 30 example sodium or potassium, an alkaline earth metal salt for example calcium or magnesium, or organic amine salt for example triethylamine.

In vivo hydrolysable esters are those pharmaceutically acceptable esters that hydrolyse in the human body to produce the parent compound. Such esters can be identified by administering, for example intravenously to a test animal, the compound under test and subsequently examining the test animal's body fluids. Suitable in vivo hydrolysable esters for carboxy include methoxymethyl and for hydroxy include acetyl.

In order to use a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof for the therapeutic treatment (including prophylactic treatment) of mammals including humans, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Therefore in another aspect the present invention provides a pharmaceutical composition which comprises a compound of the formula (I) or a pharmaceutically acceptable salt or an in vivo hydrolysable ester and pharmaceutically acceptable carrier.

The pharmaceutical compositions of this invention may be administered in standard manner for the disease condition that it is desired to treat, for example by oral, topical, parenteral, buccal, nasal, vaginal or rectal administration or by inhalation. For these purposes the compounds of this invention may be formulated by means known in the art into the form of, for example, tablets, capsules, aqueous or oily solutions, suspensions, emulsions, creams, ointments, gels, nasal sprays, suppositories, finely divided powders or aerosols for inhalation, and for parenteral use (including intravenous, intramuscular or infusion) sterile aqueous or oily solutions or suspensions or sterile emulsions.

In addition to the compounds of the present invention the pharmaceutical composition of this invention may also contain, or be co-administered (simultaneously or sequentially) with, one or more pharmacological agents of value in treating one or more disease conditions referred to hereinabove.

The pharmaceutical compositions of this invention will normally be administered to humans so that, for example, a daily dose of 0.5 to 75 mg/kg body weight (and preferably of 0.5 to 30 mg/kg body weight) is received. This daily dose may be given in divided doses as necessary, the precise amount of the compound received and the route of administration depending on the weight, age and sex of the patient being treated and on the particular disease condition being treated according to principles known in the art.

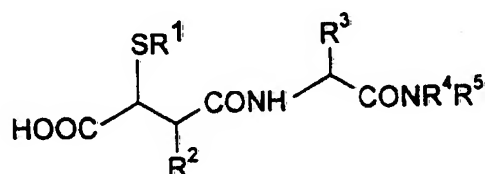
Typically unit dosage forms will contain about 1 mg to 500 mg of a compound of this invention.

Therefore in a further aspect, the present invention provides a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof for use
5 in a method of therapeutic treatment of the human or animal body.

In yet a further aspect the present invention provides a method of treating a disease condition mediated by TNF which comprises administering to a warm-blooded animal an effective amount of a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof. The present invention also provides the use of a compound
10 of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof in the preparation of a medicament for use in a disease condition mediated by TNF.

In another aspect the present invention provides a process for preparing a compound of the formula (I) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof which process comprises

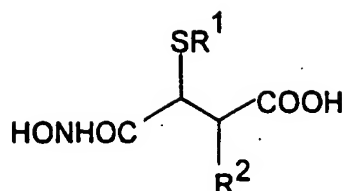
15 a) reacting a compound of the formula (III):



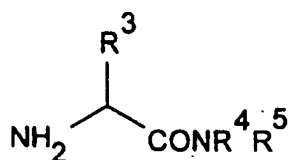
(III)

20 wherein R¹-R⁵ are as hereinbefore defined, or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine or a salt thereof; or

b) coupling a compound of the formula (IV) with a compound of the formula (V):

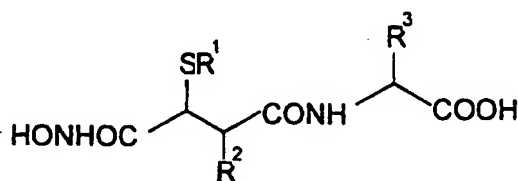


(IV)



(V)

- 5 wherein R¹-R⁵ are as hereinbefore defined, under standard peptide coupling conditions; or
 c) reacting a compound of the formula (VI) with compound of the formula (VII):



(VI)

10



(VII)

wherein R¹-R⁵ are as hereinbefore defined;

wherein any functional group is protected, if necessary, and:

- 15 i. removing any protecting groups;
 - ii. optionally forming a pharmaceutically acceptable salt or in vivo hydrolysable ester.
- Protecting groups may in general be chosen from any of the groups described in the literature or known to the skilled chemist as appropriate for the protection of the group in question, and may be introduced by conventional methods.
- 20 Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting group in question, such methods being chosen so as to effect removal of the protecting group

with minimum disturbance of groups elsewhere in the molecule.

Specific examples of protecting groups are given below for the sake of convenience, in which "lower" signifies that the group to which it is applied preferably has 1-4 carbon atoms. It will be understood that these examples are not exhaustive. Where specific examples of methods for the removal of protecting groups are given below these are similarly not exhaustive. The use of protecting groups and methods of deprotection not specifically mentioned is of course within the scope of the invention.

A carboxyl protecting group may be the residue of an ester-forming aliphatic or araliphatic alcohol or of an ester-forming silanol (the said alcohol or silanol preferably containing 1-20 carbon atoms).

Examples of carboxy protecting groups include straight or branched chain (1-12C)alkyl groups (eg isopropyl, t-butyl); lower alkoxy lower alkyl groups (eg methoxymethyl, ethoxymethyl, isobutoxymethyl); lower aliphatic acyloxy lower alkyl groups, (eg acetoxymethyl, propionyloxymethyl, butyryloxymethyl, pivaloyloxymethyl); lower alkoxycarbonyloxy lower alkyl groups (eg 1-methoxycarbonyloxyethyl, 1-ethoxycarbonyloxyethyl); aryl lower alkyl groups (eg benzyl, p-methoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, benzhydryl and phthalidyl); tri(lower alkyl)silyl groups (eg trimethylsilyl and t-butyldimethylsilyl); tri(lower alkyl)silyl lower alkyl groups (eg trimethylsilylethyl); and (2-6C)alkenyl groups (eg allyl and vinyllethyl).

Methods particularly appropriate for the removal of carboxyl protecting groups include for example acid-, base-, metal- or enzymically-catalysed hydrolysis.

Examples of hydroxyl protecting groups include lower alkyl groups (eg t-butyl), lower alkenyl groups (eg allyl); lower alkanoyl groups (eg acetyl); lower alkoxycarbonyl groups (eg t-butoxycarbonyl); lower alkenyloxycarbonyl groups (eg allyloxycarbonyl); aryl lower alkoxycarbonyl groups (eg benzoyloxycarbonyl, p-methoxybenzyloxycarbonyl, o-nitrobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl); tri lower alkylsilyl (eg trimethylsilyl, t-butyldimethylsilyl) and aryl lower alkyl (eg benzyl) groups.

Examples of amino protecting groups include formyl, aralkyl groups (eg benzyl and substituted benzyl, p-methoxybenzyl, nitrobenzyl and 2,4-dimethoxybenzyl, and triphenylmethyl); di-p-anisylmethyl and furylmethyl groups; lower alkoxycarbonyl (eg

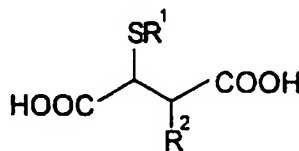
t-butoxycarbonyl); lower alkenyloxycarbonyl (eg allyloxycarbonyl); aryl lower alkoxycarbonyl groups (eg benzyloxycarbonyl, p-methoxybenzyloxycarbonyl, o-nitrobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl); trialkylsilyl (eg trimethylsilyl and t-butyl dimethylsilyl); alkylidene (eg methyldiene); benzylidene and substituted benzylidene groups.

Methods appropriate for removal of hydroxy and amino protecting groups include, for example, acid-, base-, metal- or enzymically-catalysed hydrolysis, for groups such as p-nitrobenzyloxycarbonyl, hydrogenation and for groups such as o-nitrobenzyloxycarbonyl, photolytically.

10 The hydroxylamine group (HONH-), in particular in process variants (b) and (c), is typically O-protected for example with benzyl, 4-methoxybenzyl, 2,4-dimethoxybenzyl, t-butyl or a silyl (for example trimethylsilyl) group.

The compound of the formula (III) may be reacted in the form of the acid or an activated derivative thereof such as an acid halide, acid anhydride or an 'activated' ester such as 15 as 1H-benzo[1,2,3]triazol-1-yl, 1-hydroxy-benzo[1,2,3]triazole, pentafluorophenyl or 2,4,5-trichlorophenyl in the presence of a carbodiimide. The reaction of the compound of the formula (III) and hydroxylamine is performed under standard conditions. Typically the reaction of an activated ester of a compound of the formula (III) and hydroxylamine or O-protected hydroxylamine is performed in the presence of a base, for example 2,6-lutidine, 20 (optionally in the presence of dimethylaminopyridine) or N-methylmorpholine in an anhydrous aprotic solvent, for example dimethylformamide, at a non-extreme temperature, for example in the region -30° to +25°, preferably about 0°C.

The compound of the formula (III) may be prepared by reacting a compound of the formula (VIII) with a compound of the formula (V):

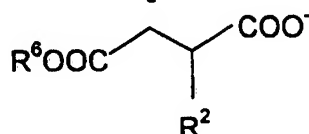


25

(VIII)

wherein R¹ and R² are as hereinbefore defined, and wherein any functional group is protected and removed as necessary, under standard peptide coupling reaction conditions.

The compounds of the formula (VIII) may be prepared by reacting a source of $-SR^1$ with a dianion (formed in situ) of the formula (IX):

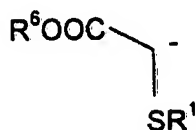


(IX)

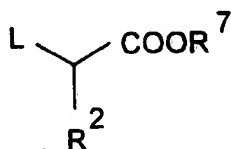
- 5 wherein R^2 is as hereinbefore defined and R^6 is a protecting group, with subsequent removal of the protecting group if necessary. The dianion may be formed in situ by the action on the corresponding methylene compound, of a strong base, for example lithium di-isopropylamide, in an anhydrous substantially inert solvent, for example tetrahydrofuran, dimethylformamide, dimethylsulfoxide or a mixture thereof, at a low temperature such as -78°C . The
- 10 corresponding methylene compounds are known in the art, for example see PCT Patent Application WO 94/21625. The source of $-SR^1$ may conveniently be a disulfide $R^1-S-S-R^1$ which is reacted in situ with the dianion of the formula (IX).

The compounds of the formula (VIII) may also be prepared by reacting a compound of the formula (X) with a compound of the formula (XI):

15



(X)



(XI)

- 20 wherein R^1 , R^2 and R^6 are as hereinbefore defined, R^7 is a protecting group and L is a leaving group, for example a methanesulphonyloxy, trifluoromethanesulphonyloxy or p-toluenesulphonyloxy group. The anion of the formula (X) may be formed in situ by the action of a strong base on the corresponding methylene compound.

The compounds of the formulae (IV) and (V) are reacted under standard peptide coupling conditions wherein any functional group is protected as necessary. The compounds of the formula (IV) may be prepared by reacting hydroxylamine with a compound of the formula (VIII), both compounds protected as necessary under conditions similar to those described above for preparing compounds of the formula (III). The compounds of the formula (V) may be prepared under standard conditions for acylation of an amine.

The compounds of the formulae (VI) and (VIII) are reacted under standard conditions for acylation of an amine with any functional groups protected as necessary. The compounds of the formula (VI) may be prepared by methods similar to those described above for preparing compounds of the formula (IV).

The following biological test methods, data and Examples serve to illustrate the present invention.

Isolated Enzyme Assay

The ability of the compounds of this invention to inhibit proTNF α convertase enzyme is assessed in an isolated enzyme assay (termed "CON2"). Partially purified proTNF α convertase enzyme is obtained from the membranes of THP-1 cells as follows. 1.5-2.0x10⁶ cells/ml THP-1 cells (initially cultured in RPMI 1640 medium + 10%(v/v) FCS, 10%(v/v) M1, 2mM L-glutamine 100IU/ml penicillin and 100 μ g/ml streptomycin) are induced in RPMI 1640 containing 1 μ g/ml LPS (E. coli O111:B4), 2mM Hydroxyurea, 50 μ g/ml silica and 1%(v/v) FCS at 37°C in a humidified (5%CO₂/95%air) incubator. After 16 hours the cells are harvested from a 5L induction by centrifugation at 640xg for 15 minutes. The cell pellets are washed once in RPMI 1640 without additive (1L per 2x10¹⁰ cells) and re-centrifuged at 640xg for 10 minutes. Cell pellets are resuspended in 10mM sodium phosphate buffer pH 7.4, containing 1mM MgCl₂, 30mM NaCl, 5 μ M PMSF, 0.02%(w/v) sodium azide (Buffer A) plus a few micrograms DNAase using 3 times the volume of buffer to packed cell pellets. A polytron homogeniser is used to lyse the cells by 5x5sec bursts with 1-2 minutes cooling between each burst. 30 ml of this homogenate is layered onto 10 mls of 41% (w/v) sucrose made in Buffer A and centrifuged at 150,000xg for 1 hour in a swing out rotor. The membrane is collected from the interphase, diluted by addition of 4 volumes Buffer A and centrifuged at 150,000xg for 20 minutes. The pellet is then resuspended in Buffer A containing 1%(w/v) Triton X-100 to a concentration of 1mg/ml

and mixed for 1 hour at 4°C. The solubilised protein is recovered by centrifugation for 30 minutes at 100,000xg at 4°C. The supernatant is applied to a 25ml gelatin-sepharose 4B column equilibrated in 10mM Tris-HCl pH 8.0, 100mM NaCl, 0.1%(w/v) Triton X-100, 200µM PMSF, 0.02%(w/v) azide, 1µM ZnCl₂ (Buffer B). After loading the column is

5 washed with Buffer B. The gelatin-sepharose flowthrough plus the first 10mls of the wash is then recycled overnight (1ml/min) on a 30ml wheatgerm-sepharose column previously equilibrated in 10mM Tris-HCl pH 8.0, 0.1%(w/v) Triton X-100, 200µM PMSF, 0.02%(w/v) azide, 1µM ZnCl₂ (Buffer C). After loading the column is washed in Buffer C and the enzyme is eluted with Buffer C containing 300mM N-Acetyl Glucosamine. The active

10 enzyme fractions are applied to a 1ml Mono Q column equilibrated in Buffer C. After loading and washing with Buffer C, enzyme is eluted using a 0-500mM NaCl gradient in Buffer C. Active enzyme fractions are pooled and used as partially purified proTNFα convertase. In all cases the active fractions are assayed using the fluorogenic synthetic peptide substrate assay described below. This enzyme preparation cleaves 21kD soluble proTNFα at the correct

15 cleavage site (Ala-Val) and enzyme activity is inhibited by matrix metalloprotease inhibitors (Gearing, A.J.H. et al., 1995, J Leukocyte Biol., 57,774-777). 4',5'-Dimethoxy-fluoresceinyl Ser.Pro.Leu.Ala.Gln.Ala.Val.Arg.Ser.Ser.Ser.Arg.Cys(4-(3-succinimid-1-yl)-fluorescein)-NH₂ the substrate is used to measure proTNFα convertase enzyme activity in CON2. It was synthesised as follows. The peptidic part of the substrate was assembled on Fmoc-NH-Rink-

20 MBHA-polystyrene resin either manually or on an automated peptide synthesiser by standard methods involving the use of Fmoc-amino acids and O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) as coupling agent with at least a 4- or 5-fold excess of Fmoc-amino acid and HBTU. Ser¹ and Pro² were double-coupled. The following side chain protection strategy was employed; Ser¹(Bu^t), Gln⁵(Trityl), Arg^{8,12}(Pmc or

25 Pbf), Ser^{9,10,11}(Trityl), Cys¹³(Trityl). Following assembly, the N-terminal Fmoc protecting group was removed by treating the Fmoc-peptidyl-resin with piperidine in DMF. The amino-peptidyl-resin so obtained was acylated by treatment for 1.5-2hr at 70°C with 1.5-2 equivalents of 4',5'-dimethoxy-fluorescein-4(5)-carboxylic acid (Khanna & Ullman, Anal Biochem, 108, 156-161, 1980) which had been preactivated with diisopropylcarbodiimide and

30 1-hydroxybenzotriazole in DMF. The dimethoxyfluoresceinyl-peptide was then simultaneously deprotected and cleaved from the resin by treatment with trifluoroacetic acid

containing 5% each of water and triethylsilane. The dimethoxyfluoresceinyl-peptide was isolated by evaporation, trituration with diethyl ether and filtration. The isolated peptide was reacted with 4-(N-maleimido)-fluorescein in DMF containing diisopropylethylamine, the product purified by RP-HPLC and finally isolated by freeze-drying from aqueous acetic acid.

5 The product was characterised by MALDI-TOF MS and amino acid analysis.

Test compounds are serially diluted in assay buffer (50mM Tris HCl, pH 7.4 containing 0.1% (w/v) Triton X-100 and 2mM CaCl_2) and 50 μl of each concentration is added to appropriate wells of a 96 well plate and 50 μl assay buffer is added to substrate alone (n=6) and substrate +enzyme (n=6) control wells. ProTNF α convertase enzyme (25 μl ; 0.0144
10 units/ml in assay buffer) is added to all wells, except substrate alone controls which receive 25 μl assay buffer. (NB: One unit of enzyme activity is defined as the convertase enzyme concentration which converts 1nMole substrate/hour).

Plates are incubated for 15 minutes at 26°C, prior to addition of 25 μl substrate (40 μM stock solution in assay buffer). Plates are then incubated at 26°C for 18 hours and read on a
15 Fluoroskan II fluorometer (plates are also read at time 0 to obtain background values). In this test, generally, compounds are of interest if they have activity below 500nM. By way of example the compound of Example 1 gave a figure of 0.8 nM.

Assessment in human cell line (THF-2)

The ability of the compounds of this invention to inhibit TNF α production is
20 assessed in THP-1 cells which are a human myelomonocytic cell line which synthesise and secrete TNF α when stimulated with lipopolysaccharide. THP-1 cells (4x10⁵ cells in 160 μl medium RPMI 1640 + bicarbonate, penicillin, streptomycin and glutamine) are incubated with 20 μl of test compounds (triplicates) in DMSO or appropriate vehicle, in a 96 well tissue culture (TC) plate, for 30 min at 37°C in a humidified (5%CO₂/95%air) incubator, prior to
25 addition of 20 μl lipopolysaccharide (LPS) (E. Coli. 0111:B4 (Sigma); final concentration 50 $\mu\text{g/ml}$). Each assay includes controls of THP-1 cells incubated with medium alone (six wells/plate) or with a standard TNF α inhibitor. The plates are then incubated for 6 hours at 37°C (humidified incubator) after which time 100 μl samples are removed from each well and transferred to a 96 well plate for storage at -70°C for subsequent analysis of TNF α
30 concentration by ELISA. In this test, generally, compounds are of interest if they have activity below 10 μM .

Assessment in whole blood assay

The ability of the compounds of this invention to inhibit TNF α production is also assessed in a human whole blood assay (HWBA). Human whole blood secretes TNF α when stimulated with LPS. This property of blood forms the basis of an assay which is used as a secondary test for compounds which profile as active in the THP-1 test. Heparinized (10Units/ml) human blood obtained from volunteers is diluted 1:5 with medium (RPMI1640 + bicarbonate, penicillin, streptomycin and glutamine) and incubated (160 μ l) with 20 μ l of test compound (triplicates), in DMSO or appropriate vehicle, for 30 min at 37°C in a humidified (5%CO₂/95%air) incubator, prior to addition of 20 μ l LPS (E. coli. 0111:B4; final concentration 10 μ g/ml). Each assay includes controls of diluted blood incubated with medium alone (6 wells/plate) or a known TNF α inhibitor as standard. The plates are then incubated for 6 hours at 37°C (humidified incubator), centrifuged (2000rpm for 10 min; 4°C), plasma harvested (50-100 μ l) and stored in 96 well plates at -70°C before subsequent analysis for TNF α concentration by ELISA. In this test, generally, compounds are of interest if they have activity below 50 μ M.

In vivo assessment

The ability of the compounds of this invention as *ex vivo* TNF α inhibitors is assessed in the rat. Briefly, groups of male Wistar Alderley Park (AP) rats (180-210g) are dosed with compound (6 rats) or drug vehicle (10 rats) by the appropriate route e.g. peroral (p.o.), intraperitoneal (i.p.), subcutaneous (s.c.). Ninety minutes later rats are sacrificed using a rising concentration of CO₂ and bled out via the posterior vena cavae into 5 Units of sodium heparin/ml blood. Blood samples are immediately placed on ice and centrifuged at 2000 rpm for 10 min at 4°C and the harvested plasmas frozen at -20°C for subsequent assay of their effect on TNF α production by LPS-stimulated human blood. The rat plasma samples are thawed and 175 μ l of each sample are added to a set format pattern in a 96U well plate. Fifty μ l of heparinized human blood is then added to each well, mixed and the plate is incubated for 30 min at 37°C (humidified incubator). LPS (25 μ l; final concentration 10 μ g/ml) is added to the wells and incubation continued for a further 5.5 hours. Control wells are incubated with 25 μ l of medium alone. Plates are then centrifuged for 10 min at 2000 rpm and 200 μ l of the supernatants are transferred to a 96 well plate and frozen at -20°C for subsequent analysis of TNF concentration by ELISA.

Data analysis by dedicated software calculates for each compound/dose:

$$\text{Percent inhibition of TNF}\alpha = \frac{\text{Mean TNF}\alpha (\text{Controls}) - \text{Mean TNF}\alpha (\text{Treated})}{\text{Mean TNF}\alpha (\text{Controls})} \times 100$$

5 Pharmacokinetic test

To evaluate the clearance properties of the compounds of this invention a sensitive ex vivo pharmacokinetic test is employed which utilises the CON2 assay to evaluate clearance rate.

This is a generic test which can be used to estimate the clearance rate of compounds across a range of species. Animals (eg, rats, marmosets) are dosed iv with a soluble formulation of compound and at subsequent time points (e.g. 5, 10, 15, 20, 30, 45, 60, 120 min) blood samples are taken from an appropriate vessel into 10U heparin. Plasma fractions are obtained following centrifugation and the plasma proteins precipitated with ethanol (70% final concentration). After 30 mins at 4°C the plasma proteins are sedimented by centrifugation and the supernatant fraction is evaporated to dryness using a Savant speed vac. The sediment is reconstituted in CON2 assay buffer and subsequently analysed for compound content using the TNF convertase assay (CON2). Briefly, a compound concentration-response curve is constructed for the compound undergoing evaluation. Serial dilutions of the reconstituted plasma extracts are assessed for activity and the amount of compound present in the original plasma sample is calculated using the concentration-response curve taking into account the total plasma dilution factor.

Test as anti-arthritic agent

Activity of a compound as an anti-arthritic is tested as follows. Acid soluble native type II collagen was shown by Trentham et al. [1] to be arthritogenic in rats; it caused polyarthritis when administered in Freund's incomplete adjuvant. This is now known as collagen-induced arthritis (CIA) and similar conditions can be induced in mice and primates. Recent studies have shown that anti-TNF monoclonal antibodies [2] and TNF receptor-IgG fusion proteins [3] ameliorate established CIA indicating that TNF plays a key role in the pathophysiology of CIA. Moreover, the remarkable efficacy reported for anti-TNF monoclonal antibodies in recent rheumatoid arthritis clinical trials indicates that TNF plays a

major role in this chronic inflammatory disease. Thus CIA in DBA/1 mice as described in references 2 and 3 is a tertiary model which can be used to demonstrate the anti-arthritis activity of a compound.

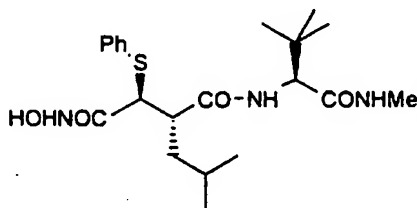
- 5 1. Trentham, D.E. et al., (1977) J. Exp. Med., 146, 857.
2. Williams, R.O. et al., (1992) Proc Natl Acad Sci, 89, 9784.
3. Williams, R.O. et al., (1995) Immunology, 84, 433.

In the examples:

10

- (a) NMR spectra were taken at 400 MHz;
- (b) DMF means dimethylformamide;
- (c) Evaporation of solvents was carried out under reduced pressure;
- (d) LDA means lithium di-isopropylamide;
- 15 (e) THF means tetrahydrofuran;
- (f) DMSO means dimethylsulphoxide;
- (g) AcOH means acetic acid.
- (h) DMAP means dimethylaminopyridine.

20

Example 1N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide

- 5 N²-[4-Hydroxy-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide (2.15 g, 5.1 mmol) was dissolved in DMF (15 ml). 1-Hydroxybenzotriazole (770 mg, 5.7 mmol) was added, followed by N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.09 g, 5.7 mmol). The mixture was stirred at room temperature for one hour. Hydroxylamine hydrochloride (529 mg, 7.6 mmol) was added immediately followed by 2,6-lutidine (830 μ l, 7.6 mmol) and DMAP (62 mg, 0.51 mmol). The resulting solution was stirred at room temperature for 3 hours. The resulting mixture was purified by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 20/80 to 60/40). Elution yielded the title compound (520 mg; yield: 24%); m.p. = 198-200°C; ¹H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.6 Hz), 0.84 (d, 3H, J= 6.6 Hz), 0.93 (s, 9H), 0.9-1.0 (m, 1H), 1.3-1.5 (m, 2H), 15 2.55 (d, 3H, J= 4.8 Hz), 3.00 (m, 1H), 3.56 (d, 1H, J= 11.3 Hz), 4.22 (d, 1H, J= 9.5 Hz), 7.15-7.4 (m, 5H), 7.79 (q, 1H, J= 4.8 Hz), 8.00 (d, 1H, J= 9.5 Hz), 9.0 (s br, 1H), 10.75 (m, 1H); MS (ESI): 446 (M + Na⁺).

- 20 N²-[4-Hydroxy-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide used as the starting material was obtained as follows:

- (i) To a stirred solution of LDA [32.8 mmol; prepared by addition of 1.6 M n-butyl lithium (20.5 ml, 32.8 mmol) in hexane to a solution of diisopropylamine (4.4 ml, 33.75 mmol) in dry THF (20 ml) at -78°C] cooled at -78°C under argon atmosphere was added 2R-isobutylbutan-25 1,4-dioic acid-4-tert-butyl ester ⁽¹⁾ (3.45 g, 15 mmol) in dry THF (15 ml) dropwise. The mixture was stirred for 90 minutes at -78°C and a solution of diphenyl disulfide (4.2 g, 19 mmol) in dry THF (15 ml) was added. The mixture was stirred for 30 minutes at -78°C, warmed to room temperature and stirred for two hours at room temperature. The solution was

cooled to -78°C and quenched by addition of methanol (6 ml). The solution was warmed to room temperature and the solvents were evaporated in vacuo. Ice was added to the residue and the mixture was acidified with 2N hydrochloric acid to pH 4. The solution was extracted with diethyl ether (3 x 100 ml). The combined organic extracts were dried over MgSO₄, filtered and
5 the solvents were removed. The residue was purified by flash chromatography on silica using petroleum ether-ethyl acetate (gradient from 8/2 to 0/10) as eluant to give a mixture of 2S-isobutyl-3(R,S)-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester (4.77g; ratio R/S: 50/50). Preparative normal phase HPLC purification using ethyl acetate-cyclohexane (5:95) as eluant gave 2S-isobutyl-3S-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester (2.3 g): ¹H-NMR
10 (CDCl₃): 0.83 (d, 3H, J= 7.3 Hz), 0.85 (d, 3H, J= 7Hz), 1.26 (m, 1H), 1.39 (s, 9H), 1.63 (m, 1H), 1.72 (m, 1H), 2.98 (m, 1H), 3.68 (d, 1H, J= 10.3 Hz), 7.28 (m, 3H), 7.50 (m, 2H).

Further elution gave the other isomer (2S-isobutyl-3R-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester) (2.2 g): ¹H-NMR (CDCl₃): 0.93 (d, 6H, J= 6.2Hz), 1.35 (s, 9H), 1.6-1.75 (m, 2H),
15 1.86 (m, 1H), 2.81 (m, 1H), 3.62 (d, 1H, J= 10.3 Hz), 7.30 (m, 3H), 7.49 (m, 2H).

⁽ⁱ⁾ British Biotech Ltd, (Crimmin, M.J.; Beckett, P.R.; Davis, M.H.), Patent WO94/21625 (1994)

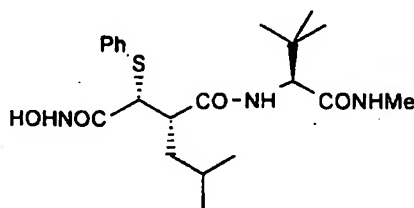
20 (ii) To 2S-isobutyl-3S-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester (2.75 g, 8.1 mmol) in DMF (16 ml) at 0°C was added successively 1-hydroxybenzotriazole (1.32 g, 9.8 mmol) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.87 g, 9.8 mmol). After 15 minutes, L-tert-leucine methylamide (1.41 g, 9.8 mmol) was added to the mixture followed by DMAP (195 mg, 1.6 mmol). The mixture was stirred at room temperature for three hours. The
25 mixture was poured into cold water and extracted with diethyl ether (2 x 100 ml). The combined organic layers were washed with saturated sodium bicarbonate, brine and dried over MgSO₄. The solvents were evaporated in vacuo and the residue was purified by flash chromatography on silica using ethyl acetate-petroleum ether (3:7) as eluant to give N²-[2S-isobutyl-3S-phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide. (3.0 g, yield:
30 79%) as a white solid: ¹H-NMR (CDCl₃): 0.84 (d, 3H, J= 6.6 Hz), 0.90 (d, 3H, J= 6.2 Hz), 1.06 (s, 9H), 1.17 (m, 1H), 1.34 (s, 9H), 1.45 (m, 1H), 1.74 (m, 1H), 2.67 (m, 1H), 2.80 (d,

3H, J= 4.8 Hz), 3.71 (d, 1H, J= 11 Hz), 4.27 (d, 1H, J= 9.2 Hz), 5.95 (s br, 1H), 6.45 (d, 1H, J= 9.2 Hz), 7.27 (m, 3H), 7.47 (m, 2H).

(iii) Trifluoroacetic acid (8.3 ml) was added dropwise to a solution of N²-[2S-isobutyl-3S-phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (3.0 g, 6.05 mmol) in dry dichloromethane (19 ml). The solution was stirred at 0°C overnight. The solvents were evaporated in vacuo. The residue was taken up in toluene and the solvent was removed in vacuo (three times) to give white crystals which were washed with pentane and dried in vacuo to yield N²-[4-hydroxy-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide (2.56 g, 97%): ¹H-NMR (DMSO d-6): 0.79 (d, 1H, J= 6.6 Hz), 0.87 (d, 1H, J= 6.6 Hz), 0.96 (s, 9H), 1.06 (m, 1H), 1.40 (m, 1H), 1.56 (m, 1H), 2.57 (d, 3H, J= 4.4 Hz), 3.03 (m, 1H), 3.62 (d, 1H, J= 11 Hz), 4.26 (d, 1H, J= 9.2 Hz), 7.43-7.24 (m, 5H), 7.86 (q, 1H, J= 4.4 Hz), 8.13 (d br, 1H, J= 9.2 Hz).

15 Example 2

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide



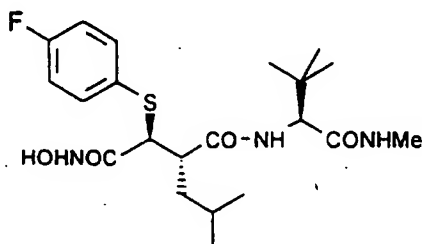
N²-[4-Hydroxy-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide (170 mg, 0.41 mmol) was dissolved in DMF (3.5 ml) and cooled at 0°C. 1-Hydroxybenzotriazole (73 mg, 0.54 mmol) was added, followed by N-methylmorpholine (60 µl, 0.54 mmol) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (103 mg, 0.54 mmol). The mixture was stirred at 0°C for one hour. A mixture of hydroxylamine hydrochloride (58 mg, 0.83 mmol) and N-methyl morpholine (91 µl, 0.83 mmol) in DMF (1.5 ml) was added. The resulting solution was stirred at 0°C for one hour then at room temperature overnight. The solvents were evaporated in vacuo and the residue was purified by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 20/80 to 60/40). Elution yielded the compound of Example 1 (55 mg; yield: 32%). Further elution using the

same solvent gave N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide (49 mg, yield: 29%): m.p.= 194-198°C; ¹H-NMR (DMSO d-6): 0.75 (d, 3H, J= 6.6 Hz), 0.79 (d, 3H, J= 6.6 Hz), 0.87 (s, 9H), 1.3-1.6 (m, 3H), 2.55 (d, 3H, J= 4.8 Hz), 2.88 (m, 1H), 3.62 (d, 1H, J= 9.5 Hz), 4.12 (d, 1H, J= 9.5 Hz), 7.2-7.5 (m, 5H), 7.69 (d, 1H, J= 9.5 Hz), 7.78 (q, 1H, J= 4.8 Hz), 8.86 (s, 1H), 10.75 (m, 1H); MS (ESI): 446 (M + Na⁺).

N²-[4-Hydroxy-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide used as the starting material was obtained as follows:

- 10 (i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3R-phenylthiobutan-1,4-dioic acid-4-tert-butyl ester (478 mg, 1.4 mmol) there was obtained N²-[2S-isobutyl-3R-phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (540 mg, yield: 78%) as a white foam: ¹H-NMR (CDCl₃): 0.87 (d, 1H, J= 6.6 Hz), 0.88 (d, 1H, J= 6.6 Hz), 0.99 (s, 9H), 1.33 (s, 9H), 1.51 (m, 1H), 1.64 (m, 1H), 1.79 (m, 1H), 2.64 (m, 1H), 2.78 (d, 3H, J= 5.1 Hz), 3.73 (d, 1H, J= 9.9 Hz), 4.14 (d, 1H, J= 9.2 Hz), 5.88 (q, 1H, J= 5.1 Hz), 6.56 (d, 1H, J= 9.2 Hz), 7.30 (m, 3H), 7.49 (m, 2H).

- (ii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3R-phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (490 mg) there was
20 obtained N²-[4-hydroxy-2S-isobutyl 3R-phenylthiosuccinyl]-L-tert-leucine-N¹-methanamide (460 mg) as a white solid: ¹H-NMR (CDCl₃): 0.84 (d, 3H, J= 6.2 Hz), 0.87 (d, 3H, J= 6.2 Hz), 1.06 (s, 9H), 1.55-1.80 (m, 3H), 2.86 (d, 3H, J= 5.1 Hz), 2.97 (m, 1H), 3.84 (d, 1H, J= 6.6 Hz), 4.38 (d, 1H; J= 9.5 Hz), 6.28 (s br, 1H), 7.32 (m, 3H), 7.50 (m, 2H).

Example 3**N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide**

5 In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (530 mg, 1.24 mmol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (240 mg, 36%) as a white solid:
 10 m.p.= 195-197°C; ¹H-NMR (DMSO d-6): 0.77 (d, 3H, J= 6.2 Hz), 0.85 (d, 3H, J= 6.6 Hz), 0.96 (s, 9H), 1.0 (m, 1H), 1.37 (m, 2H), 2.57 (d, 3H, J= 4.4 Hz), 3.01 (m, 1H), 3.46 (d, 1H, J= 11.4 Hz), 4.26 (d, 1H, J= 9.1 Hz), 7.17 (dd, 2H, J= J'= 8.8 Hz), 7.41 (dd, 2H, J=5.5 Hz, J'= 8.8 Hz), 7.81 (q, 1H, J= 4.4 Hz), 8.03 (d, 1H, J= 9.1 Hz), 8.98 (s br, 1H); 10.55 (s br, 1H); MS (ESI): 464 (M + Na⁺).

15

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.15 g, 5 mmol) and di-(4-fluorophenyl) disulfide⁽²⁾ (1.4 g), there was obtained 2S-isobutyl-3S-(4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester:
 20 ¹H-NMR (CDCl₃): 0.90 (d, 1H, J= 6.6 Hz), 0.92 (d, 1H, J= 6.6 Hz), 1.25 (m, 1H), 1.41 (s, 9H), 1.75-1.55 (m, 2H), 2.94 (m, 1H), 3.56 (d, 1H, J= 10.6 Hz), 7.00 (dd, 2H, J=J'= 8.8 Hz), 7.50 (dd, 2H, J= 8.8 Hz, J'= 5.1 Hz) and the other isomer: 2S-isobutyl-3R-(4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester: ¹H-NMR (CDCl₃): 0.93 (m, 6H), 1.37 (s, 9H), 1.9-1.3 (m, 3H), 2.77 (m, 1H), 3.54 (d, 1H, J= 10.3 Hz), 7.02 (dd, 2H, J=J'= 8.8 Hz), 7.49 (dd, 2H,
 25 J= 8.8 Hz, J'= 5.1 Hz).

(ii) In a manner analogous to that described in Example 1 (ii) except that dichloromethane was used instead of DMF as a solvent, from 2S-isobutyl-3S-(4-fluorophenyl)thiobutan-1,4-dioic

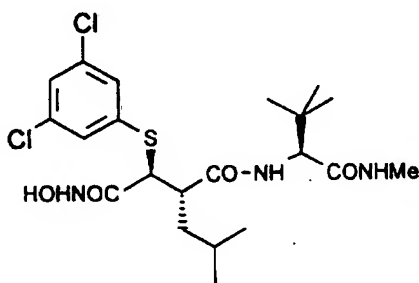
acid-4-tert-butyl ester (800 mg, 2.24 mmol), there was obtained N²-[2S-isobutyl-3S-(4-fluorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (810 mg, 75%) as a white solid: ¹H-NMR (CDCl₃): 0.84 (d, 3H, J= 6.6 Hz), 0.89 (d, 3H, J= 6.6 Hz), 1.06 (s, 9H), 1.15 (m, 1H), 1.37 (s, 9H), 1.45 (m, 1H), 1.72 (m, 1H), 2.65 (m, 1H), 2.81 (d, 3H, J= 4.6 Hz), 3.61 (d, 1H, J= 11 Hz), 4.27 (d, 1H, J= 9.2 Hz), 5.88 (s br, 1H), 6.44 (d, 1H, J= 9.2 Hz), 6.98 (dd, J=J'= 8.8 Hz), 7.46 (dd, 2H, J= 5.1 Hz, J'= 8.8 Hz).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(4-fluorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (800 mg, 1.66 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (700 mg) as a white solid: ¹H-NMR (DMSO d-6): 0.78 (d, 1H, J=6.6 Hz), 0.87 (d, 1H, J= 6.6 Hz), 0.97 (s, 9H), 1.05 (m, 1H), 1.6-1.35 (m, 2H), 2.58 (d, 1H, J= 4.4 Hz), 3.03 (m, 1H), 3.54 (d, 1H, J= 11.4 Hz), 4.28 (d, 1H, J= 9.5 Hz), 7.18 (m, 2H), 7.47 (dd, 2H, J= 5.5 Hz, J'= 8.8 Hz), 7.88 (q, 1H, J= 4.4 Hz), 8.13 (d, 1H, J= 9.5 Hz).

(2) Unless stated, the disulfides are prepared from the corresponding commercially available thiols by oxidation with iodine.

Example 4

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(3,5-dichlorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide



In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2S-isobutyl-3S-(3,5-dichlorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (770 mg) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,5-dichlorophenyl)-

thiosuccinyl]-L-tert-leucine-N¹-methanamide (80 mg, 11%) as a white solid; m.p.= 199-202°C:
¹H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.2 Hz), 0.86 (d, 3H, J= 6.2 Hz), 0.91 (s, 9H), 1.01 (m, 1H), 1.37 (m, 2H), 2.56 (d, 3H, J= 4.4 Hz), 3.10 (m, 1H), 3.68 (d, 1H, J= 11 Hz), 4.24 (d, 1H, J= 9.5Hz), 7.42 (d, 2H, J= 1.8 Hz), 7.46 (t, 1H, J= 1.8 Hz), 7.85 (q, 1H, J= 4.4 Hz), 8.13 (d, 5 1H, J= 9.5 Hz), 9.1 (s br, 1H), 10.6 (m, 1H); MS (ESI): 518 (M{³⁷Cl, ³⁷Cl} + Na⁺), 516 (M{³⁵Cl, ³⁷Cl} + Na⁺), 514 (M{³⁵Cl, ³⁵Cl} + Na⁺).

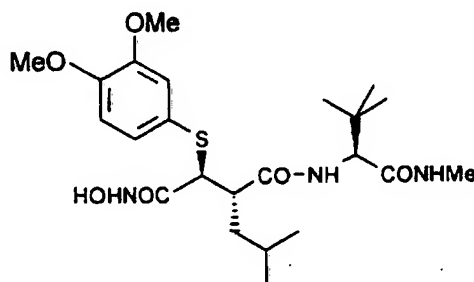
The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic
10 acid-4-tert-butyl ester (1.5 g, 6.5 mmol) and di-(3,5-dichlorophenyl) disulfide⁽²⁾ (2.54 g), there was obtained 2S-isobutyl-3S-(3,5-dichlorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.0 g): ¹H-NMR (CDCl₃): 0.92 (d, 3H, J= 6.6 Hz), 0.94 (d, 3H, J= 6.6 Hz), 1.25 (m, 1H), 1.43 (s, 9H), 1.8-1.5 (m, 2H), 2.97 (m, 1H), 3.70 (d, 1H, J= 10.3 Hz), 7.26 (1H), 7.38 (d, 2H, J= 1.8 Hz) and the other isomer: 2S-isobutyl-3R-(3,5-dichlorophenyl)thiobutan-1,4-dioic acid-4-tert-
15 butyl ester (300 mg): ¹H-NMR (CDCl₃): 0.94 (m, 6H), 1.40 (s, 9H), 1.8 1.5 (m, 3H), 2.83 (m, 1H), 3.69 (d, 1H, J= 10.2 Hz), 7.28 (1H, t, J= 1.8 Hz), 7.39 (d, 2H, J= 1.8 Hz).

(ii) In a manner analogous to that described in Example 1 (ii) except that dichloromethane was used instead of DMF as a solvent, from 2S-isobutyl-3S-(3,5-dichlorophenyl)thiobutan-1,4-
20 dioic acid-4-tert-butyl ester (1 g, 2.45 mmol), there was obtained N²-[2S-isobutyl-3S-(3,5-dichlorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (1.0 g, 77%) as a white foam.

MS (ESI): 559 (M{³⁷Cl, ³⁷Cl} + Na⁺), 557 (M{³⁵Cl, ³⁷Cl} + Na⁺), 555 (M{³⁵Cl, ³⁵Cl} + Na⁺)

25 (iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(3,5-dichlorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (1.0 g, 1.9 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(3,5-dichlorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (775 mg) as a white solid: ¹H-NMR (DMSO d-6): 0.80 (d, 3H, J= 6.6 Hz), 0.87 (d, 3H, J= 6.2 Hz), 0.93 (s, 9H), 1.1 (m, 1H), 1.40 (m, 1H), 1.57 (m, 1H), 2.56 (d, 30 3H, J= 4.4 Hz), 3.09 (m, 1H), 3.72 (d, 1H, J= 10.6 Hz), 4.26 (d, 1H, J=9.6 Hz), 7.45 (s, 2H), 7.52 (s, 1H), 7.90 (q, 1H, J= 4.4 Hz), 8.21 (d br, 1H, J= 9.6 Hz)

Example 5N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide

5

In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (450 mg) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,4-dimethoxyphenyl)-thiosuccinyl]-L-tert-leucine-N¹-methylamide (170 mg) as a white solid: m.p.= 218-220°C; ¹H-NMR (DMSO d-6): 0.77 (d, 3H, J= 6.2 Hz), 0.85 (d, 3H, J= 6.2 Hz), 0.98 (s, 9H), 1.0 (m, 1H), 1.40 (m, 2H), 2.58 (d, 3H, J= 4.4 Hz), 2.98 (m, 1H), 3.37 (d, 1H, J= 11.4 Hz), 3.75 (s, 3H), 3.76 (s, 3H), 4.29 (d, 1H, J= 9.5 Hz), 6.88 (m, 2H), 6.99 (s, 1H), 7.81 (q, 1H, J= 4.4 Hz), 7.99 (d, 1H, J= 9.5 Hz), 8.98 (s br, 1H); 10.53 (s br, 1H); MS (ESI): 506 (M + Na⁺).

15 The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.0 g, 4.34 mmol) and di-(3,4-dimethoxyphenyl) disulfide⁽²⁾ (2.0 g), there was obtained 2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.05 g): ¹H-NMR (CDCl₃): 0.90 (d, 3H, J= 6.6 Hz), 0.91 (d, 3H, J= 6.6 Hz), 1.26 (m, 1H), 1.42 (s, 9H), 1.75-1.55 (m, 2H), 2.96 (m, 1H), 3.54 (d, 1H, J= 10.3 Hz), 3.86 (s, 3H), 3.87 (s, 3H), 6.78 (d, 1H, J= 8.1 Hz), 7.1 (m, 2H) and the other isomer: 2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (550 mg): ¹H-NMR (CDCl₃): 0.94 (d, 6H, J= 6.6 Hz), 1.38 (s, 9H), 1.75-1.55 (m, 2H), 1.86 (m, 1H), 2.78 (m, 1H), 3.50 (d, 1H, J= 10.3 Hz), 3.87 (s, 3H), 3.88 (s, 3H), 6.80 (d, 1H, J= 8.1 Hz), 7.02 (d, 1H, J= 2.2 Hz), 25 7.09 (dd, 1H, J= 2.2 Hz, J'= 8.1 Hz).

- 29 -

(ii) In a manner analogous to that described in Example 1 (ii) except that DMAP was replaced by 2,6-lutidine (1 equivalent), from 2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1 g, 2.45 mmol), there was obtained N²-[2S-isobutyl-3S-(3,4-dimethoxyphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (1.1 g, 84%) as
 5 a white foam.

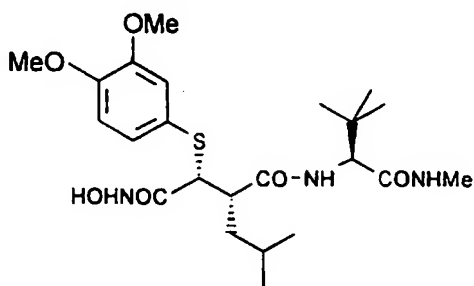
MS (ESI): 547 (M + Na⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(3,4-dimethoxyphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (900 mg, 1.7
 10 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(3,4-dimethoxyphenyl)thio-succinyl]-L-tert-leucine-N¹-methylamide (795 mg) as a white solid: MS (ESI): 469 (M + H⁺), 491 (M + Na⁺).

Example 6

15

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide



In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide
 20 (450 mg) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (170 mg) as a white solid.
¹H-NMR (DMSO d-6): 0.79 (d, 3H, J= 6.6 Hz), 0.82 (d, 3H, J= 6.2 Hz), 0.89 (s, 9H), 1.35-1.50 (m, 2H), 1.65 (m, 1H), 2.57 (d, 3H, J= 4.4 Hz), 2.82 (m, 1H), 3.45 (d, 1H, J= 9.9 Hz),
 25 3.77 (s, 3H), 3.79 (s, 3H), 4.13 (d, 1H, J= 9.2Hz), 6.93 (d, 1H, J= 8.4 Hz), 7.04 (m, 2H), 7.73 (d, 1H, J= 9.2 Hz), 7.79 (s br, 1H), 8.86 (s, 1H); 10.65 (s br, 1H); MS (ESI): 506 (M + Na⁺).

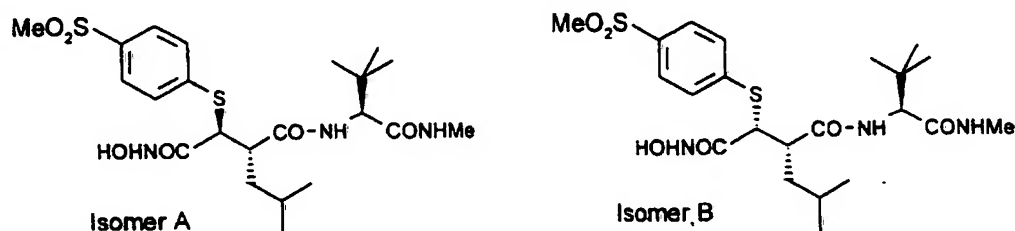
The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (ii) except that DMAP was replaced by 2,6-lutidine (1 equivalent), from 2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (480 mg, 1.2 mmol), there was obtained N²-[2S-isobutyl-3R-(3,4-dimethoxyphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (555 mg, 88%) as a white foam : MS (ESI): 547 (M + Na⁺).

(ii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3R-(3,4-dimethoxyphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (500 mg, 0.95 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (256 mg) as a white solid.
MS (ESI): 469 (M + H⁺), 491 (M + Na⁺).

Example 7

N²-[4-(N-Hydroxyamino)-2S-isobutyl-S-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (Isomer A) and
N²-[4-(N-Hydroxyamino)-2S-isobutyl-R-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (Isomer B)



In a manner analogous to that described in the first paragraph of Example 1 except that O-(trimethylsilyl)hydroxylamine was used instead of hydroxylamine hydrochloride, from the mixture of N²-[4-hydroxy-2S-isobutyl 3(R/S)-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (450 mg, 1:1 mixture) there was obtained N²-[4-(N-hydroxyamino)

2S-isobutyl-3S-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (Isomer A) (105 mg) as a white solid after purification of the crude mixture by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 45/55): m.p.= 222-224°C: ¹H-NMR (DMSO d-6): 0.83 (d, 3H, J= 6.6 Hz), 0.91 (d, 3H, J= 6.2 Hz), 0.94 (s, 9H), 1.08 (m, 1H), 1.44 (m, 1H), 1.55 (m, 1H), 2.60 (d, 3H, J= 4.8 Hz), 3.15 (m, 1H), 3.26 (s, 3H), 3.86 (d, 1H, J= 11.3 Hz), 4.23 (d, 1H, J= 9.2 Hz), 7.61 (d, 2H, J= 8.8 Hz), 7.83 (m, 3H), 8.1 (d, 1H, J= 9.2 Hz), 9.15 (s, 1H), 10.85 (s br, 1H); MS (ESI): 524 (M + Na⁺).

Further elution with methanol and water/1%AcOH (45:55) gave N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (Isomer B) (77 mg) as a white solid: ¹H-NMR (DMSO d-6): 0.82 (d, 3H, J= 6.6 Hz), 0.85 (d, 3H, J= 6.6 Hz), 0.93 (s, 9H), 1.48 (m, 3H), 2.62 (d, 3H, J= 4.4 Hz), 3.03 (m, 1H), 3.27 (s, 3H), 3.41 (d, 1H, J= 9.2 Hz), 4.21 (d, 1H, J= 9.2 Hz), 7.71 (d, 2H, J= 8.4 Hz), 7.89 (m, 4H), 8.97 (s, 1H), 10.9 (s br, 1H); MS (ESI): 524 (M + Na⁺).

15

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) except that di-(4-(methylsulfonyl)phenyl) disulfide was dissolved in DMF instead of THF, from 2R-isobutyl-butan-1,4-dioic acid-4-tert-butyl ester (1.31 g) and di-(4-(methylsulfonyl)phenyl) disulfide⁽³⁾ (2.15 g), there was obtained a mixture of 2S-isobutyl-3(R/S)-(4-(methylsulfonyl)phenyl)thio-butan-1,4-dioic acid-4-tert-butyl ester (800 mg, 1:1 mixture): ¹H-NMR (CDCl₃): 0.94 (m, 6H), 1.38 (s, 9H, isomer B), 1.42 (s, 9H, isomer A), 1.8-1.3 (m, 3H), 2.89 (m, 1H, isomer B), 3.01 (m, 1H, isomer A), 3.05 (s, 3H), 3.85 (d, 1H, J= 10.2 Hz), 7.63 (m, 2H), 7.86 (m, 2H).

(ii) In a manner analogous to that described in Example 1 (ii) except that DMAP was replaced by 2,6 lutidine (1 equivalent), from the mixture of 2S-isobutyl-3(R/S)-(4-(methylsulfonyl)phenyl)thio-butan-1,4-dioic acid-4-tert-butyl ester (800 mg, 1:1 mixture), there was obtained a mixture of N²-[2S-isobutyl 3(R/S)-(4-(methylsulfonyl)phenyl)thio 4-tert-butyl oxy succinyl]-L-tert-leucine-N¹-methylamide (800 mg, 78%) as a white foam.

MS (ESI): 565 (M + Na⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from the mixture of N²-[2S-isobutyl 3(R/S)-4-(methylsulfonyl)phenylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (800 mg, 1.4 mmol, 1:1 mixture) there was obtained a mixture of N²-[4-hydroxy-2S-isobutyl-3(R/S)-4-(methylsulfonyl)phenylthio succinyl]-L-tert-leucine-N¹-methylamide (500 mg) as a white solid: MS (ESI): 509 (M + Na⁺).

⁽³⁾ Bordwell, F.G. et al.; J. Am. Chem. Soc., 1953, 75, 6019

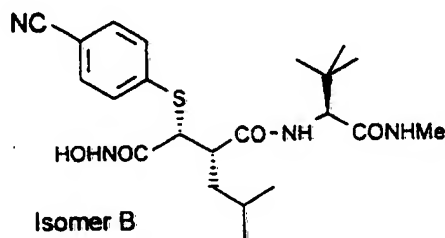
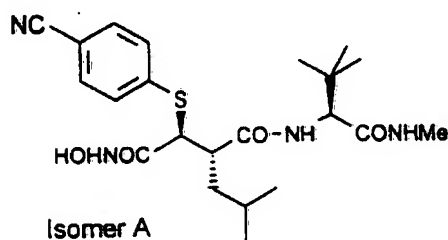
Example 8

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N²-[4-(N-Hydroxyamino)-2S-isobutyl-S-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (Isomer A) and

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3R-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (Isomer B)

15



In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2S-isobutyl-3S-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (300 mg, 0.7 mmol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-cyanophenyl)-thiosuccinyl]-L-tert-leucine-N¹-methylamide (68 mg, 22%) as a white solid (isomer A): m.p. = 238-241°C: ¹H-NMR (DMSO d-6): 0.78 (d, 3H, J = 6.6 Hz), 0.86 (d, 3H, J = 6.6 Hz), 0.88 (s, 3H); 0.97-1.08 (m, 1H), 1.3-1.53 (m, 2H), 2.55 (d, 3H, J = 4.8 Hz), 3.06-3.15 (m, 1H), 3.80 (d, 1H, J = 11 Hz), 4.17 (d, 1H, J = 9.2 Hz), 7.51 (d, 2H, J = 8.8 Hz), 7.73 (d, 2H, J = 8.5 Hz), 7.75-7.83 (m, 1H), 8.09 (d, 1H, J = 9.2 Hz), 9.05-9.15 (m, 1H), 10.75-10.90 (m, 1H) and N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (20 mg) as a white solid (isomer B) formed by epimerisation during the reaction: ¹H-NMR

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(DMSO d-6): 0.76 (d, 3H, J= 6 Hz), 0.80 (d, 3H, J = 6 Hz), 0.88 (s, 9H), 1.38-1.6 (m, 3H), 2.56 (d, 3H, J= 4.4 Hz), 2.97-3.04 (m, 1H), 3.88 (d, 1H, J= 9.5 Hz), 4.15 (d, 1H, J= 9.5 Hz), 7.60 (d, 2H, J= 8 Hz), 7.80 (d, 2H, J= 8.4 Hz), 7.75-7.85 (m, 2H), 8.94 (s, 1H), 10.85 (s, 1H).

5 The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.5 g, 6.5 mmol) and di-(4-cyanophenyl) disulfide⁽⁴⁾ (1.8 g), there was obtained 2S-isobutyl-3S-(4-cyanophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.2 g):

¹H-NMR (CDCl₃): 0.92 (m, 6H), 1.3 (m, 1H), 1.41(s, 9H), 1.8-1.55 (m, 2H), 2.99 (m, 1H),
10 3.82 (d, 1H, J= 9.9 Hz), 7.56 (m, 4H) and the other isomer: 2S-isobutyl-3R-(4-cyanophenyl)-thiobutan-1,4-dioic acid-4-tert-butyl ester (300 mg): ¹H-NMR (CDCl₃): 0.92 (d, 6H, J= 5.9 Hz), 1.37 (s, 9H), 1.8-1.5 (m, 3H), 2.89 (m, 1H), 3.82 (d, 1H, J= 10.2 Hz), 7.57 (m, 4H).

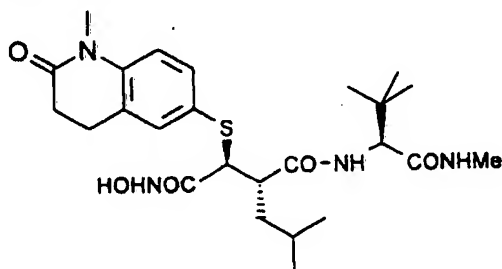
(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(4-
15 cyanophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.1 g, 3 mmol), there was obtained N²-[2S-isobutyl-3S-(4-cyanophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (1.09 g, 74%) as a white foam: MS (ESI): 512 (M + Na⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(4-
20 cyanophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (1.09 g, 2.2 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (737 mg) as a white solid: MS (ESI): 434 (M + H⁺), 456 (M + Na⁺).

⁽⁴⁾ Krishnamurthy, S. and al.; J. Org. Chem., 1989, 54, 4458

Example 9

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide



5

In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (360 mg, 0.73 mol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (75 mg, 20%) as a white solid: m.p.= 235°C (decomposition): ¹H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.6 Hz), 0.85 (d, 3H, J= 6.2 Hz), 0.97 (s, 9H), 0.95-1.05 (m, 1H), 1.3-1.5 (m, 2H), 2.57 (d, 3H, J= 4.8 Hz), 2.5-2.6 (m, 2H), 2.83 (m, 2H), 3.00 (m, 1H), 3.24 (s, 3H), 3.46 (d, 1H, J= 11.3 Hz), 4.26 (d, 1H, J= 9.2 Hz), 7.02 (d, 1H, J= 8.5 Hz), 7.2 (s, 1H); 7.26 (d, 1H, J= 8.5 Hz), 7.8-7.85 (m, 1H), 8.00 (d, 1H, J= 9 Hz), 8.9-9.1 (m, 1H); 10.5-10.8 (m, 1H);

10 MS (ESI): 529 (M + Na⁺).

15

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.3 g, 5.65 mmol) and di-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl) disulfide⁽⁵⁾ (2.8 g), there was obtained 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (950 mg) after purification on C18 preparative HPLC eluting with aqueous ammonium carbonate (0.2%)-acetonitrile (gradient from 100:0 to 72.5:27.5): MS (ESI): 444 (M + Na⁺).

20

Further elution gave the other isomer: 2S-isobutyl-3R-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (603 mg): MS (ESI): 444 (M + Na⁺).

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(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl 3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (390 mg, 0.92 mmol), there was obtained N²-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (250 mg,) as a white foam
 5 after C18 preparative HPLC eluting with aqueous ammonium carbonate (0.2%)-acetonitrile (gradient from 90:10 to 50:50): MS (ESI): 570 (M + Na⁺).

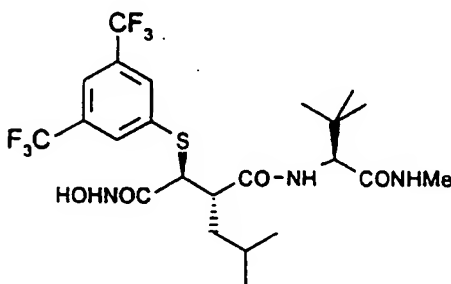
(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-
 10 methylamide (410 mg, 0.75 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (370 mg) as a white solid: ¹H-NMR (CDCl₃): 0.84 (d, 3H, J= 6.6 Hz), 0.88 (d, 3H, J= 6.3 Hz), 1.03 (s, 9H), 1.34 (m, 1H), 1.46 (m, 1H), 1.70 (m, 1H), 2.63 (m, 2H), 2.82 (d, 3H, J= 4.7 Hz), 2.86 (m, 2H), 2.93 (m, 1H), 3.81 (d, 1H, J= 9.2 Hz), 4.37 (d, 1H, J= 9.6 Hz), 6.38 (d, 1H, J= 8.4 Hz), 7.29 (d, 1H, J= 2.2 Hz), 7.40 (dd, 1H, J= 8.4 Hz, J'= 2.2 Hz), 7.7 (s br, 1H).
 15

⁽³⁾ Imperial Chemical Industries PLC, ICI-Pharma S.A.; (Bruneau, P.); EPA-462812

Example 10

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N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide



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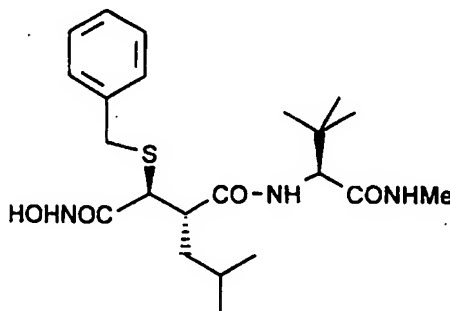
In a manner analogous to that described in the first paragraph of Example 1, from N²-[4-hydroxy-2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (500 mg, 0.91 mmol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (42 mg) as a
5 white solid: m.p.= 225-230°C; ¹H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (s br, 12H), 1.0-1.1 (m, 1H), 1.3-1.5 (m, 2H), 2.56 (d, 3H, J= 4.8 Hz), 3.1-3.2 (m, 1H), 3.77 (d, 1H, J= 11.3 Hz), 4.25 (d, 1H, J= 9.5 Hz), 7.85-7.90 (m, 1H), 7.94 (s, 1H), 8.04 (s, 2H), 8.15-8.21 (m, 1H), 9.0-9.12 (m, 1H), 10.8-10.92 (m, 1H); MS (ESI): 582 (M + Na⁺).

10 The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.5 g, 6.5 mmol) and di-[3,5-di-(trifluoromethyl)phenyl] disulfide⁽²⁾ (3.66 g), there was obtained 2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.3 g) after purification on C18 preparative HPLC eluting with
15 methanol and water/1%AcOH (gradient from 20/80 to 70/30): MS (ESI): 497 (M + Na⁺); the other isomer 2S-isobutyl-3R-(3,5-di-trifluoromethyl)phenylthiobutan-1,4-dioic acid 4-tert-butyl ester which was formed in the reaction as a minor component was not isolated.

(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(3,5-di-
20 (trifluoromethyl)phenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.3 g, 2.7 mmol), there was obtained N²-[2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (1.2 g) as a white foam: MS (ESI): 623 (M + Na⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(3,5-
25 di-(trifluoromethyl)phenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (1.1 g, 1.8 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(3,5-trifluoromethyl)phenyl)thio succinyl]-L-tert-leucine-N¹-methylamide (990 mg) as a white solid : MS (ESI): 545 (M + H⁺), 567 (M + Na⁺).

Example 11N²-[4-(N-Hydroxyamino)-2S-isobutyl]-3S-benzylthiosuccinyl]-L-tert-leucine-N¹-methanamide

- 5 In a manner analogous to that described in the first paragraph of Example 1 except that O-(t-butyl)dimethylsilyl)hydroxylamine was used instead of hydroxylamine hydrochloride and no base was added, from N²-[4-hydroxy-2S-isobutyl]-3S-benzylthiosuccinyl]-L-tert-leucine-N¹-methanamide (150 mg, 0.35 mmol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl]-3S-benzylthiosuccinyl]-L-tert-leucine-N¹-methanamide (78 mg) as a white solid after addition
 10 of 1 ml of 1N hydrochloric acid to the crude mixture at the end of the reaction and purification of this mixture on C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 60/40) m.p.= 200-205°C; ¹H-NMR (DMSO d-6): 0.75 (d, 3H, J= 6.2 Hz), 0.85 (d, 3H, J= 6.2 Hz), 0.95 (s, 9H), 1.00 (m, 1H), 1.45-1.30 (m, 2H), 2.55 (d, 3H, J= 4.4 Hz), 3.10 (m, 1H), 3.20 (d, 1H, J= 15 Hz), 3.83 (d, 1H, J= 12 Hz), 4.04 (d, 1H, J= 12 Hz), 4.26 (d, 1H, J= 9.5 Hz), 7.30-7.20 (m, 5H), 7.85 (d, 1H, J= 4.4 Hz), 8.05 (d, 1H, J= 9.5 Hz), 9.0 (s br, 1H), 10.64 (s br, 1H); MS (ESI): 460 (M + Na⁺).

The starting material was prepared as follows:

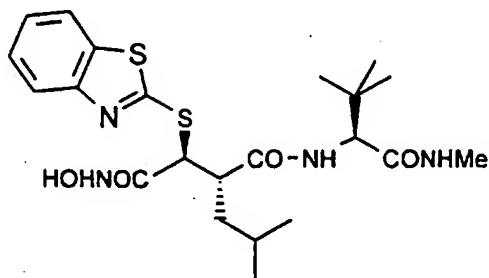
- (i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.5 g, 6.5 mmol) and dibenzyl disulfide (3.66 g), there was obtained
 20 2S-isobutyl-3R-benzylthiobutan-1,4-dioic acid-4-tert-butyl ester (1.48 g): ¹H-NMR (CDCl₃): 0.82 (d, 3H, J=6 Hz), 0.85 (d, 3H, J=5.8 Hz); 1.35 (m, 1H), 1.65-1.50 (m, 2H), 2.70 (m, 1H), 3.10 (d, 1H, J= 10 Hz), 3.84(d, 1H, J= 11.7 Hz), 3.87 (d, 1H, J= 11.7 Hz), 7.35-7.20 (m, 5H).

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(ii) To a stirred solution of LDA [9.24 mmol; prepared by addition of 2.5 M n-butyl lithium (3.69 ml, 9.24 mmol) in hexane to a solution of diisopropylamine (1.29 ml, 9.24 mmol) in dry THF (5 ml) at -78°C] cooled at -78°C under an argon atmosphere was added 2S-isobutyl-3R-benzylthiobutan-1,4-dioic acid-4-tert-butyl ester (1.48 g, 4.20 mmol) in dry THF (10 ml) dropwise. The mixture was stirred for 15 minutes at -78°C, warmed to room temperature and stirred for two hours at room temperature. The solution was cooled to -78°C and quenched at -78°C by addition of methanol (3 ml). The solution was warmed to room temperature and the solvents were evaporated in vacuo. The residue was dissolved in dichloromethane and was washed successively with 1N hydrochloric acid and brine. The organic layer was dried over MgSO₄, filtered and the solvents were removed. The residue was purified by C18 preparative HPLC using methanol-aqueous 0.2% ammonium carbonate (60:40) as eluant. The fractions were collected, acidified to pH 2 with 2N hydrochloric acid and extracted with ether (2x100 ml). The organic layer was dried over MgSO₄ and evaporated to give 2S-isobutyl-3S-benzylthiobutan-1,4-dioic acid-4-tert-butyl ester (250 mg) as an oil: ¹H-NMR (CDCl₃): 0.84 (d, 3H, J= 6 Hz), 0.86 (d, 3H, J= 6 Hz), 1.20 (m, 1H), 1.50 (s, 9H), 1.65 (m, 2H), 2.92 (m, 1H), 3.16 (d, 1H, J= 10 Hz), 3.85 (s, 2H), 7.35-7.20 (m, 5H).

(iii) In a manner analogous to that described in Example 1 (ii) except that 2,6-lutidine (1 equivalent) was used instead of DMAP, from 2S-isobutyl-3S-benzylthiobutan-1,4-dioic acid-4-tert-butyl ester (225 mg, 0.64 mmol), there was obtained N²-[2S-isobutyl-3S-benzylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (254 mg, 83 %) as a white foam.
MS (ESI): 501 (M + Na⁺).

(iv) In a manner analogous to that described in example 1 (iii), from N²-[2S-isobutyl-3S-benzylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (250 mg, 0.52 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-benzylthiosuccinyl]-L-tert-leucine-N¹-methylamide (165 mg) as a white solid: MS (ESI): 423 (M + H⁺), 445 (M + Na⁺).

Example 12N²-[4-(N-Hydroxyamino)-2S-isobutyl 3S-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide

5

In a manner analogous to that described in the first paragraph of Example 1 except that O-(tert-butyl)dimethylsilyl)hydroxylamine (1.5 equivalents) was used instead of hydroxylamine hydrochloride and 1.3 equivalents of 2,6-lutidine was used, from N²-[4-hydroxy-2S-isobutyl 3(R/S)-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (600 mg, 1.29 mmol)

10 there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (125 mg) as a white solid after addition of 2 ml of 3N hydrochloric acid to the crude mixture at the end of the reaction and purification of this mixture on C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 60/40): ¹H-NMR (DMSO d-6): 0.81 (d, 3H, J= 6.6 Hz), 0.87 (d, 3H, J=

15 6.6 Hz), 0.92 (s, 9H), 1.09 (m, 1H), 1.39 (m, 1H), 1.59 (m, 1H), 2.56 (d, 3H, J= 4.4 Hz), 3.20 (m, 1H), 4.17 (d, 1H, J= 9.9 Hz), 4.35 (d, 1H, J= 11 Hz), 7.39 (t, 1H, J= 7.7 Hz), 7.50 (t, 1H, J= 7.7 Hz), 7.81 (m, 1H), 7.89 (d, 1H, J= 7.7 Hz), 8.01 (d, 1H, J= 7.7 Hz), 8.09 (d, 1H, J=9.2Hz), 9.13 (s, 1H), 10.95 (s, 1H); MS (ESI): 481 (M + H⁺), 503 (M + Na⁺). Further elution gave N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-

20 N¹-methylamide.

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) except that the solution of the dianion in THF cooled to -78°C was transferred through a double-ended needle into a slurry of

25 di-(benzothiazol-2-yl) disulfide⁽²⁾ in DMSO-THF (20 ml: 10 ml) cooled at 0°C and the mixture was stirred at 0°C for one hour, from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (1.15g,

5 mmol) and di-(benzothiazol-2-yl) disulfide⁽²⁾ (1.4 g), there was obtained a mixture of 2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (830 mg, 1:1 mixture) after purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (70/30): ¹H-NMR (CDCl₃): 0.96-0.87 (m, 6H), 1.41 and 1.42 (s, 9H), 1.9-1.6 (m, 3H), 3.20 and 3.29 (m, 1H), 4.77 (d, 1H, J= 8 Hz) and 4.79 (d, 1H, J= 7.6 Hz), 7.34 (m, 1H), 7.44 (m, 1H), 7.77 (m, 1H), 7.89 (m, 1H)

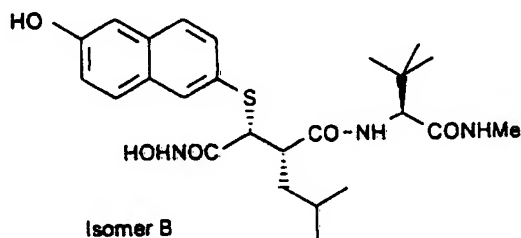
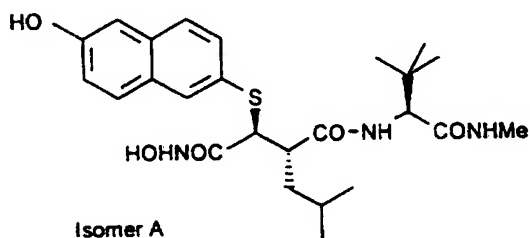
(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (800 mg, 2.0 mmol), there was obtained N²-[2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (983 mg, 94%) as a white foam: MS (ESI): 522 (M + H⁺), 544 (M + Na⁺), 560 (M + K⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (900 mg, 1.72 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3(R/S)-(benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (725 mg, 91%) as a white solid: MS (ESI): 466 (M + H⁺), 488 (M + Na⁺).

20 Example 13

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (Isomer A) and

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3R-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (Isomer B) .



- In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyltrimethylsilyl)hydroxylamine was used instead of hydroxylamine hydrochloride and no base was added, from N²-[4-hydroxy-2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (650 mg, 1.26 mmol) there was obtained after treatment of the
- 5 crude reaction mixture with HCl (2N, 10 drops) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/ 1% AcOH (gradient from 30/70 to 60/40) N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (Isomer A) (65 mg, 10%) as a white solid: ¹H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.6 Hz), 0.84 (d, 3H, J= 6.6 Hz), 0.95 (s, 9H), 1.0 (m, 1H), 1.35-1.55 (m, 2H), 2.55
- 10 (d, 3H, J= 4.7 Hz), 3.05 (m, 1H), 3.56 (d, 1H, J= 11.4 Hz), 4.26 (d, 1H, J= 9.1 Hz), 7.1 (m, 2H), 7.36 (d, 1H, J= 8.4 Hz), 7.61 (d, 1H, J= 8.8 Hz), 7.68 (d, 1H, J= 9.5 Hz), 7.77 (s, 1H), 7.8 (s br, 1H), 8.03 (d, 1H, J= 9.1 Hz), 8.93 (s br, 1H), 9.81 (s, 1H), 10.58 (s br, 1H); MS (ESI): 512 (M + Na⁺).
- 15 Further elution with methanol and water/ 1% AcOH (60/40) gave N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (Isomer B) (30 mg, 5%) as a white solid formed by epimerisation during the reaction: ¹H-NMR (DMSO d-6): 0.75 (d, 3H, J= 6.6 Hz), 0.80 (d, 3H, J= 6.6 Hz), 0.86 (s, 9H), 1.4 (m, 1H), 1.5 (m, 1H), 1.65 (m, 1H), 2.55 (d, 3H, J= 4.7 Hz), 2.88 (m, 1H), 3.61 (d, 1H, J= 9.5 Hz), 4.12 (d,
- 20 1H, J= 9.1 Hz), 7.1 (m, 2H), 7.45 (d, 1H, J= 8.8 Hz), 7.67 (d, 1H, J= 8.8 Hz), 7.74 (d, 1H, J= 9.9 Hz), 7.75 (m, 1H), 7.78 (m, 1H), 7.9 (s br, 1H), 8.85 (s br, 1H), 9.86 (s, 1H), 10.8 (s br, 1H); MS (ESI): 490 (M + H⁺), 512 (M + Na⁺).

The starting material was prepared as follows:

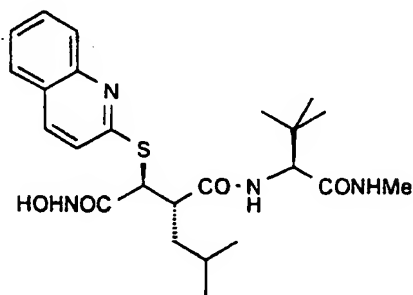
- 25 (i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid 4-tert-butyl ester (1.92 g, 8.4 mmol) and di-(6-acetoxynaphth-2-yl) disulfide⁽⁶⁾ (4.0 g), there was obtained 2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (1.52g, 41%): ¹H-NMR (CDCl₃): 0.93 (d, 3H, J= 6.2 Hz), 0.94 (d, 3H, J= 6.2 Hz), 1.33 (s, 9H), 1.62-1.7 (m, 1H), 1.7-1.76 (m, 1H), 1.86-1.93 (m, 1H), 2.35 (s, 3H), 2.85 (m, 1H), 3.72
- 30 (d, 1H, J= 10.2 Hz), 7.23-7.26 (m, 1H), 7.53-7.56 (m, 2H), 7.73 (d, 1H, J= 8.4 Hz), 7.78 (d, 1H, J= 8.8 Hz), 7.99 (s br, 1H); MS (ESI): 469 (M + Na⁺); the other isomer 2S-isobutyl-3R-(6-

acetoxynaphth-2-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester which was formed in the reaction as a minor component was not isolated.

(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (1.5 g, 3.36 mmol), there was obtained N²-[2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (1.26 g, 65%) as an off-white solid: ¹H-NMR (CDCl₃): 0.87 (d, 3H, J= 6.6 Hz), 0.89 (d, 3H, J= 6.6 Hz), 0.98 (s, 9H), 1.30 (s, 9H), 1.53 (m, 1H), 1.65 (m, 1H), 1.86 (m, 1H), 2.35 (s, 3H), 2.7 (m, 1H), 2.76 (d, 3H, J= 4.4 Hz), 3.79 (d, 1H, J= 10.2 Hz), 4.28 (d, 1H, J= 9.5 Hz), 6.58-6.66 (m, 2H), 7.25 (m, 1H), 7.53-7.57 (m, 2H), 7.73 (d, 1H, J= 8.4 Hz), 7.77 (d, 1H, J= 9.1 Hz), 7.99 (s, 1H); MS (ESI): 573 (M + H⁺), 595 (M + Na⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methanamide (780 mg, 1.36 mmol) there was obtained after purification by flash chromatography on silica using ethyl acetate-methanol (4:1) as eluant N²-[4-hydroxy-2S-isobutyl-3S-(6-acetoxynaphth-2-yl)thio succinyl]-L-tert-leucine-N¹-methanamide (650 mg, 92%) as a white solid: ¹H-NMR (DMSO d-6): 0.78 (d, 3H, J= 5.8 Hz), 0.82 (d, 3H, J= 5.5 Hz), 0.92 (s, 9H), 1.55-1.65 (m, 3H), 2.32 (s, 3H), 2.62 (d, 3H, J= 4.4 Hz), 2.8 (m, 1H), 3.88 (d, 1H, J= 7.3 Hz), 4.03 (m, 1H), 7.3 (dd, 1H, J= 2.2 Hz, J'= 8.9 Hz), 7.56 (dd, 1H, J= 1.8 Hz, J'= 8.8 Hz), 7.63 (d, 1H, J= 2 Hz), 7.78 (m, 1H), 7.8-7.88 (m, 3H), 7.98 (s, 1H); MS (ESI): 539 (M + Na⁺), 555 (M + K⁺).

⁽⁶⁾Prepared by acetylation using the standard method (acetyl chloride, triethylamine in THF) from the corresponding commercially available disulfide.

Example 14**N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide**

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In a manner analogous to that described in the first paragraph of Example 1 except that O-(t-butyl)dimethylsilyl)hydroxylamine (1.3 equivalent) was used instead of hydroxylamine hydrochloride, 2,6-lutidine (1.3 equivalent) and DMAP (0.1 equivalent) were used as a base, from N²-[4-hydroxy-2S-isobutyl-3(R/S)-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N¹-

10 methylamide (400 mg, 0.87 mmol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (135 mg) as a white solid after purification of the crude mixture by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 60/40): m.p.= 188-190°C; ¹H-NMR (DMSO d-6): 0.82 (d, 3H, J= 6.6 Hz), 0.88 (d, 3H, J= 6.6 Hz), 0.92 (s, 9H), 1.16 (m, 1H),
 15 1.42 (m, 1H), 1.62 (m, 1H), 2.55 (d, 3H, J= 4.4 Hz), 3.19 (m, 1H), 4.14 (d, 1H, J= 9.2 Hz), 4.54 (d, 1H, J= 11.4 Hz), 7.30 (d, 1H, J= 8.8 Hz), 7.52 (t, 1H, J= 7.3 Hz), 7.76 (m, 2H), 7.90 (d, 1H, J= 8.8 Hz), 8.16-8.04 (m, 3H), 9.05 (s br, 1H), 10.75 (s br, 1H); MS (ESI): 475 (M + H⁺), 497 (M + Na⁺).

20 Further elution gave N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide contaminated with minor impurities.

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic
 25 acid-4-tert-butyl ester (1.1 g, 4.8 mmol) and di-(quinolin-2-yl) disulfide⁽²⁾ (1.4 g), there was

obtained 2S-isobutyl-3(R/S)-(quinolin-2-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.14 g, 68 %): MS (ESI): 412 (M + Na⁺).

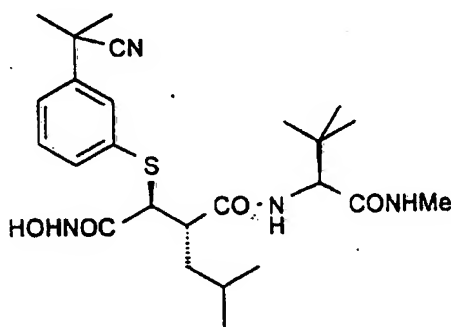
(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3(R/S)-(quinolin-2-yl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.08 g, 2.8 mmol), there was obtained N²-[2S-isobutyl-3(R/S)-(quinolin-2-yl)thio-4-tert-butyloxy-succinyl]-L-tert-leucine-N¹-methylamide (1.16 g, 80%, 4:6 mixture) as a white foam.

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3(R/S)-(quinolin-2-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (800 mg, 1.55 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3(R/S)-(quinolin-2-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (410 mg) as a white solid: MS (ESI): 482 (M + Na⁺).

Example 15

15

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N¹-methylamide



In a manner analogous to that described in the first paragraph of Example 1 except that O-(t-butyl)dimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride and 2,6-lutidine (1 equivalent) was used as a base, from N²-[4-hydroxy-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N¹-methylamide (410 mg, 0.86 mmol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N¹-methylamide (170 mg, 40%) as a white solid after addition of 2 ml of 2N hydrochloric acid to the crude mixture at the end of

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the reaction and purification of this mixture on C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 20/80 to 65/35): ¹H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (d, 3H, J= 6.6 Hz), 0.94 (s, 9H), 1.01 (m, 1H), 1.37 (m, 1H), 1.47 (m, 1H), 1.69 (s, 6H), 2.57 (d, 3H, J= 4.4 Hz), 3.04 (m, 1H), 3.62 (d, 1H, J= 11.4 Hz), 4.25 (d, 1H, J= 9.2 Hz), 7.44-7.35 (m, 4H), 7.81 (m, 1H), 8.04 (d, 1H, J= 9.2 Hz), 9.05 (s, 1H), 10.72 (s, 1H); MS (ESI): 513 (M + Na⁺).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i) , from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (0.91 g, 3.9 mmol) and di-[3-(1-cyano-1-methylethyl)phenyl] disulfide⁽²⁾ (1.6 g, 4.5 mmol), there was obtained 2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiobutan-1,4-dioic acid-4-tert-butyl ester (565 mg, 36 %): MS (ESI): 428 (M + Na⁺). Further elution gave 2S-isobutyl-3R-[3-(1-cyano-1-methylethyl)phenyl]thiobutan-1,4-dioic acid-4-tert-butyl ester.

15

(ii) In a manner analogous to that described in Example 1 (ii) except that no DMAP was added, from 2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiobutan-1,4-dioic acid-4-tert-butyl ester (565 mg, 1.4 mmol), there was obtained N²-[2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (495 mg, 67%) as a white foam: MS (ESI): 554 (M + Na⁺).

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(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (492 mg, 0.93 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine-N¹-methylamide (440 mg, 99%) as a white solid: MS (ESI): 498 (M + Na⁺).

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(iv) Di-[3-(1-cyano-1-methylethyl)phenyl] disulfide was prepared as follows:

1) alkylation of (3-bromophenyl)acetonitrile with excess sodium hydride/methyl iodide in THF gave 2-(3-bromophenyl) 2-methylpropionitrile (yield: 100%)

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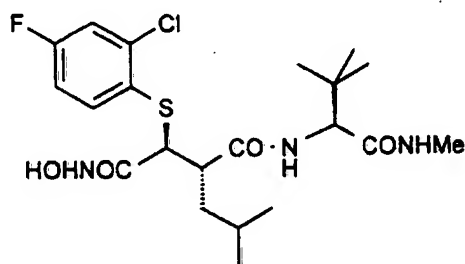
2) 2-(3-(tert-butylthio)phenyl) 2-methylpropionitrile was obtained from 2-(3-bromophenyl) 2-methylpropionitrile by reaction with tert-butyl mercaptan (1.3 eq.), potassium tert-butoxide (1.35 eq.) in DMSO at 80°C in the presence of Pd(PPh₃)₄ (0.05 eq.) (yield: 37%)

3) 2-(3-mercaptophenyl) 2-methylpropionitrile was obtained from 2-(3-(tert-butylthio)phenyl) 2-methylpropionitrile by reaction with trifluoroacetic acid (1.7 eq.) and trifluoromethylsulfonic acid (1 eq.) in thioanisole at room temperature (yield 82%)

4) oxidation of 2-(3-mercaptophenyl) 2-methyl propionitrile in DMSO gave di-[3-(1-cyano-1-methylethyl)phenyl] disulfide (yield: 73%)

10 Example 16

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide



- 15 In a manner analogous to that described in the first paragraph of Example 1 except that O-(t-butyl)dimethylsilyl)hydroxylamine (1.1 equivalent) was used instead of hydroxylamine hydrochloride and 2,6-lutidine (1 equivalent) was used as a base, from N²-[4-hydroxy-2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (1 g, 2.1 mmol) there was obtained N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(2-chloro-4-fluoro-
- 20 phenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide (451 mg, 45%) as a white solid after purification of the crude mixture by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 20/80 to 65/35): ¹H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (d, 3H, J= 6.6 Hz), 0.92 (s, 9H), 1.03 (m, 1H), 1.37 (m, 1H), 1.49 (m, 1H), 2.56 (d, 3H, J= 4.8 Hz), 3.69 (d, 1H, J= 11 Hz), 4.20 (d, 1H, J= 9.5 Hz), 7.23 (td, 1H, J_t=
- 25 8.8 Hz, J_d= 2.9 Hz), 7.46 (dd, 1H, J= 2.9 Hz, J'= 8.8 Hz), 7.80 (dd, 1H, J= 4 Hz, J'= 8.8 Hz), 9.03 (s, 1H), 10.72 (s, 1H); MS (ESI): 498 (M ³⁵Cl + Na⁺), 500 (M ³⁷Cl + Na⁺).

The starting material was prepared as follows:

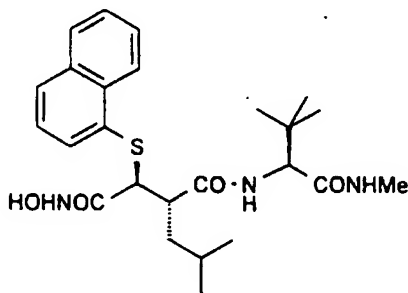
(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (2.7 g, 11.8 mmol) and di-[2-chloro-4-fluorophenyl] disulfide⁽²⁾ (4.2 g, 13 mmol), there was obtained 2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (2.5 g, 54 %): MS (ESI): 413 (M ³⁵Cl + Na⁺), 415 (M ³⁷Cl + Na⁺). Further elution gave 2S-isobutyl-3R-(2-chloro-4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester.

(ii) In a manner analogous to that described in Example 1 (ii) except that no DMAP was added, from 2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (2.5 g, 6.4 mmol), there was obtained N²-[2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (2.38 g, 72%) as a white foam. MS (ESI): 539 (M ³⁵Cl + Na⁺), 541 (M ³⁷Cl + Na⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (2.3 g, 4.4 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thio succinyl]-L-tert-leucine-N¹-methylamide (2.0 g, 97%) as a white solid: MS (ESI): 483 (M ³⁵Cl + Na⁺), 485 (M ³⁷Cl + Na⁺).

Example 17

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(naphth-1-yl)thiosuccinyl]-L-tert-leucine-N¹-methylamide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(*t*-butyldimethylsilyl)hydroxylamine (1.3 equivalents) was used instead of hydroxylamine hydrochloride and 2,6-lutidine (1.5 equivalents) was used, from N²-[4-hydroxy-2*S*-isobutyl-3*S*-(naphth-1-yl)thiosuccinyl]-L-*tert*-leucine-N¹-methanamide (1.0 g, 2.18 mmol) there was
5 obtained after treatment of the crude reaction mixture with HCl (2*N*, 1.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of acetonitrile and water/1% AcOH (gradient from 20/80 to 50/50), N²-[4-(*N*-hydroxyamino)-2*S*-isobutyl-3*S*-(naphth-1-yl)thiosuccinyl]-L-*tert*-leucine-N¹-methanamide (240 mg, 24%) as a white solid: ¹H-NMR (DMSO *d*-6): 0.76 (d, 3H, *J* = 6.2 Hz), 0.87 (d, 3H, *J* = 6.2 Hz), 0.97 (s, 9H), 1.0 (m, 1H),
10 1.35-1.55 (m, 2H), 2.57 (d, 3H, *J* = 4.0 Hz), 3.15 (m, 1H), 3.61 (d, 1H, *J* = 11.4 Hz), 4.31 (d, 1H, *J* = 9.1 Hz), 7.45 (m, 1H), 7.53 (m, 2H), 7.69 (d, 1H, *J* = 7.3 Hz), 7.83 (q, 1H, *J* = 4.0 Hz), 7.87 (d, 1H, *J* = 8.0 Hz), 7.93 (m, 1H), 8.09 (d, 1H, *J* = 9.1 Hz), 8.32 (m, 1H), 8.88 (s, 1H), 10.43 (s br, 1H); MS (ESI): 496 (M + Na⁺).

15 The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2*R*-isobutylbutan-1,4-dioic acid-4-*tert*-butyl ester (2.0 g, 8.7 mmol) and di-(naphth-1-yl) disulfide⁽⁷⁾ (3.0 g), there was obtained 2*S*-isobutyl-3*S*-(naphth-1-yl)thiobutan-1,4-dioic acid-4-*tert*-butyl ester (1.3 g, 41%); MS (EI): 388 (M +); the other isomer 2*S*-isobutyl-3*R*-(naphth-1-yl)thiobutan-1,4-dioic acid-
20 4-*tert*-butyl ester which was formed in the reaction as a minor component was not isolated.

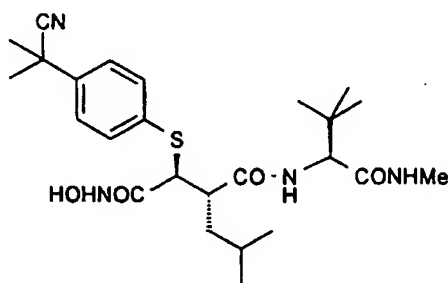
(ii) In a manner analogous to that described in Example 1 (ii), from 2*S*-isobutyl-3*S*-(naphth-1-yl)thiobutan-1,4-dioic acid-4-*tert*-butyl ester (1.3 g, 3.3 mmol), there was obtained N²-[2*S*-isobutyl-3*S*-(naphth-1-yl)thio-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methanamide (1.26 g,
25 74%) as a white solid: MS (ESI): 537 (M + Na⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2*S*-isobutyl-3*S*-(naphth-1-yl)thio-4-*tert*-butyloxysuccinyl]-L-*tert*-leucine-N¹-methanamide (1.2 g, 2.3 mmol) there was obtained N²-[4-hydroxy-2*S*-isobutyl-3*S*-(naphth-1-yl)thiosuccinyl]-L-*tert*-leucine-
30 N¹-methanamide (1.05 g, 100%): MS (ESI): 459 (M + H⁺).

⁽⁷⁾Prepared by oxidation of the commercially available thiol in DMSO.

Example 18

5 N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N²-[4-hydroxy-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)-thiosuccinyl]-L-tert-leucine-N¹-methanamide (1.4 g, 2.9 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 3.0 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 20/80 to 60/40), N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)-thiosuccinyl]-L-tert-leucine-N¹-methanamide (140 mg, 10%) as a white solid: m.p. 233-236°C: ¹H-NMR (DMSO d-6): 0.77 (d, 3H, J= 6.2 Hz), 0.85 (d, 3H, J= 6.2 Hz), 0.95 (s, 9H), 1.0 (m, 1H), 1.3-1.5 (m, 2H), 1.68 (s, 6H), 2.56 (d, 3H, J= 4.4 Hz), 3.05 (m, 1H), 3.58 (d, 1H, J= 11.4 Hz), 4.24 (d, 1H, J= 9.1 Hz), 7.42 (m, 4H), 7.81 (q, 1H, J=4.4 Hz), 8.03 (d, 1H, J= 9.1 Hz), 9.02 (s br, 1H), 10.68 (s br, 1H); MS (ESI): 513 (M + Na⁺), 529 (M + K⁺). The other isomer N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-(1-cyano-1-methylethyl)phenyl)thiosuccinyl]-L-tert-leucine-N¹-methanamide which was formed in the reaction was not separated from the first isomer and was isolated as a mixture (710 mg, 51%) therewith.

The starting material was prepared as follows:

- 25 (i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid 4-tert-butyl ester (2.5 g, 10.8 mmol) and di-4-(1-cyano-1-methylethyl)phenyl disulfide⁽⁸⁾

(4.2 g), there was obtained 2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thiobutan-1,4-dioic acid 4-tert-butyl ester (1.6g, 38%): ¹H-NMR (CDCl₃): 0.91 (dd, 6H, J= 6.6 Hz), 1.35 (m, 1H), 1.40 (s, 9H), 1.63 (m, 1H), 1.71 (s, 6H), 1.73 (m, 1H), 2.97 (m, 1H), 3.66 (d, 1H, J= 10.3 Hz), 7.46 (m, 4H); the other isomer 2S-isobutyl-3R-(4-(1-cyano-1-methylethyl)-
5 phenyl)thiobutan-1,4-dioic acid 4-tert-butyl ester which was formed in the reaction as a minor component was not isolated.

(ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thiobutan-1,4-dioic acid-4-tert-butyl ester (1.6 g, 3.9 mmol),
10 there was obtained N²-[2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thio-4-tert-butyloxy-succinyl]-L-tert-leucine-N¹-methylamide (1.6 g, 77%) as a gum: MS (ESI): 554 (M + Na⁺).

(iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (1.2
15 g, 2.3 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)-phenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (1.4 g, 100%) as a solid: ¹H-NMR (DMSO d-6): 0.79 (d, 3H, J= 6.6 Hz), 0.88 (d, 3H, J= 6.6 Hz), 0.96 (s, 9H), 1.06 (m, 1H), 1.4 (m, 1H), 1.55 (m, 1H), 1.68 (s, 6H), 2.58 (d, 3H, J= 4.4 Hz), 3.06 (m, 1H), 3.64 (d, 1H, J= 11.4 Hz), 3.7 (m br 1H), 4.26 (d, 1H, J= 9.1 Hz), 7.46 (m, 4H), 7.87 (q, 1H, J= 4.4 Hz), 8.13 (d, 1H,
20 J= 9.1 Hz).

(⁸)Di-4-(1-cyano-1-methylethyl)phenyl disulfide was prepared as follows:

1) alkylation of 4-bromophenylacetonitrile with excess sodium hydride/methyl iodide in THF gave 2-(4-bromophenyl)-2-methylpropionitrile (yield: 90%)

25 2) 2-(4-tert-butylthiophenyl)-2-methylpropionitrile was obtained from 2-(4-bromophenyl)-2-methylpropionitrile by reaction with tert-butyl mercaptan (1.1 eq.), potassium tert-butoxide (1.35 eq.) in DMSO at 70°C in the presence of Pd(PPh₃)₄ (0.03 eq.) (yield: 40%)

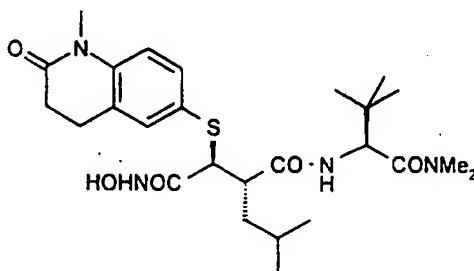
3) 2-(4-mercaptophenyl)-2-methylpropionitrile was obtained from 2-(4-tert-butylthiophenyl)-2-methylpropionitrile by reaction with trifluoroacetic acid (1.7 eq.) and
30 trifluoromethylsulfonic acid (1 eq.) in thioanisole at room temperature (yield 77%)

4) oxidation of 2-(4-mercaptophenyl)-2-methylpropionitrile in DMSO gave di-4-(1'-cyano-1'-methylethyl)phenyl disulfide (yield: 94%)

Example 19

5

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-dimethylamide



- 10 In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyl)dimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N²-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-dimethylamide (450 mg, 0.89 mmol) there was obtained after treatment of the crude reaction mixture with HCl (6N, 0.2 ml) and
- 15 purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 20/80 to 60/40), N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-dimethylamide (274 mg, 59%) as a white solid: m.p. 240-242°C: ¹H-NMR (DMSO d-6): 0.76 (d, 3H, J= 6.6 Hz), 0.83 (d, 3H, J= 6.6 Hz), 1.0 (s, 9H), 1.0 (m, 1H), 1.3-1.5 (m, 2H), 2.53 (m, 2H), 2.82 (s, 3H), 2.83 (m, 2H), 3.05 (m, 1H), 3.09 (s, 3H), 3.24 (s, 3H), 3.45 (d, 1H, J= 11.4 Hz), 4.81 (d, 1H, J= 9.2 Hz), 7.03 (d, 1H, J= 8.8 Hz), 7.22 (s, 1H), 7.27 (dd, 1H, J=1.8 Hz, J=8.8 Hz), 8.09 (d, 1H, J= 9.2 Hz), 8.96 (s br, 1H), 10.61 (s br, 1H); MS (ESI): 543 (M + Na⁺).
- 20

The starting material was prepared as follows:

25

- (i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (400 mg, 0.95

mmol), described in Example 9, and L-tert-leucine dimethylamide⁽⁹⁾ (165mg, 1.04 mmol) there was obtained N²-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butylloxysuccinyl]-L-tert-leucine-N¹-dimethylamide (500 mg, 94%) as a white solid:
MS (ESI): 584 (M + Na⁺).

5

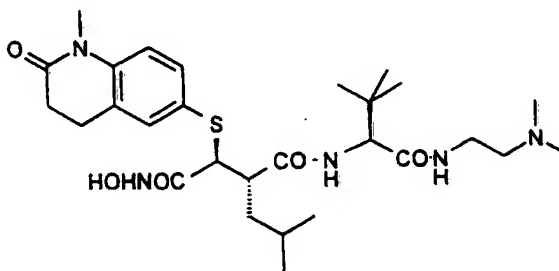
(ii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butylloxysuccinyl]-L-tert-leucine-N¹-dimethylamide (500 mg, 0.89 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-dimethylamide
10 (450 mg, 100%) as a solid: ¹H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (d, 3H, J= 6.6 Hz), 1.0 (s, 9H), 1.25 (m, 1H), 1.3-1.6 (m, 2H), 2.53 (m, 2H), 2.82 (s, 3H), 2.83 (m, 2H), 3.05 (m, 1H), 3.1 (s, 3H), 3.24 (s, 3H), 3.53 (d, 1H, J= 11.4 Hz), 4.6 (s br, 1H), 4.86 (d, 1H, J= 9.1 Hz), 7.06 (d, 1H, J= 8.8 Hz), 7.26 (s, 1H), 7.33 (m, 1H), 8.19 (d, 1H, J= 9.1 Hz).

15 ⁽⁹⁾ L-tert-leucine dimethylamide was prepared by the reaction of L-tert-leucine with triphosgene to give 3-(S)-tert-butyl oxazolidine-1,4-dione which was then treated with a saturated solution of dimethylamine in ether.

Example 20

20

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-(2-dimethylamino)ethylamide



25

In a manner analogous to that described in the first paragraph of Example 1, except that O-(*t*-butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N²-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-(2-dimethylamino)ethylamide (310 mg, 0.56 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 0.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 15/85 to 50/50), N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-(2-dimethylamino)ethylamide (123 mg, 39%) as a white solid: m.p. 152-158°C; ¹H-NMR (DMSO d-6): 0.77 (d, 3H, J = 6.6 Hz), 0.86 (d, 3H, J = 6.6 Hz), 0.99 (s, 9H), 1.1 (m, 1H), 1.3-1.5 (m, 2H), 2.5-2.57 (m, 8H), 2.7-2.75 (m, 2H), 2.83 (m, 2H), 3.04 (m, 1H), 3.24 (s, 3H), 3.33-3.4 (m, 2H), 3.47 (d, 1H, J = 11.4 Hz), 4.24 (d, 1H, J = 8.8 Hz), 7.02 (d, 1H, J = 8.4 Hz), 7.22 (s, 1H), 7.26 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz), 8.1 (m, 2H), 8.98 (s br, 1H), 10.57 (s br, 1H); MS (ESI): 564 (M + H⁺).

15

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (400 mg, 0.95 mmol), described in Example 9, and L-tert-leucine (2-dimethylamino)ethylamide⁽¹⁰⁾ (210mg, 1.04 mmol) there was obtained N²-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-(2-dimethylamino)ethylamide (369 mg, 64%) as a white solid: MS (ESI): 605 (M + H⁺).

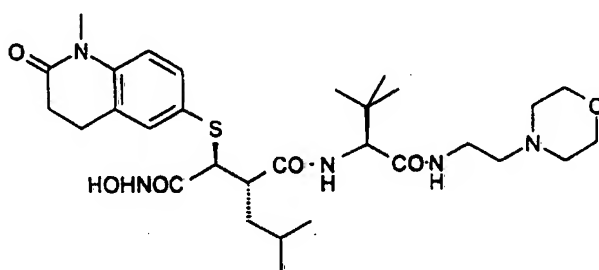
25 (ii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-(2-dimethylamino)ethylamide (324 mg, 0.56 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-(2-dimethylamino)ethylamide (430 mg, 100%) as a cream solid: MS (ESI): 548 (M⁺).

30

⁽¹⁰⁾ L-tert-leucine 2-(dimethylamino)ethylamide was prepared by the reaction of L-tert-leucine with triphosgene to give 3-(S)-tert-butyloxazolidine-1,4-dione which was then treated with N,N-dimethyl ethylenediamine.

5 Example 21

N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-[2-(4-morpholino)ethyl]amide



10

In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyl)dimethylsilylhydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N²-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-[2-(4-morpholino)ethyl]amide (237 mg, 0.4 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 0.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 15/85 to 50/50), N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-[2-(4-morpholino)ethyl]amide (85 mg, 35%) as a white solid: m.p. 156-158°C ;

20 ¹H-NMR (DMSO d-6 + TFA) : 0.78 (d, 3H, J = 6.3 Hz), 0.87 (d, 3H, J = 6.3 Hz), 1.0 (s, 9H), 1.0 (m, 1H), 1.41 (m, 2H), 2.51 (m, 2H), 2.84 (m, 2H), 3.08-3.24 (m, 5H), 3.24 (s, 3H), 3.4-3.5 (m, 4H), 3.45 (d, 1H, J = 11.3 Hz), 3.65 (m, 2H), 4.0 (m, 2H), 4.12 (m, 1H), 7.01 (d, 1H, J = 8.5 Hz), 7.21 (d, 1H, J = 2.2 Hz), 7.26 (dd, 1H, J = 2.2 Hz, J = 8.5 Hz), 8.18 (d, 1H, J = 7.8 Hz); MS (ESI) : 606 (M + H⁺).

25

The starting material was prepared as follows:

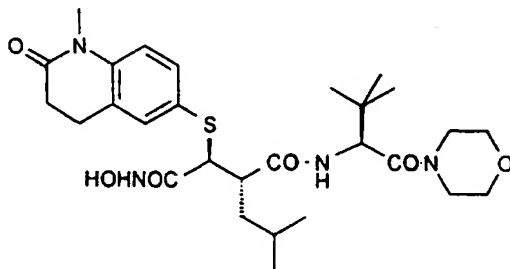
(i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiobutan-1,4-dioic acid 4-tert-butyl ester (300 mg, 0.71 mmol), described in Example 9, and L-tert-leucine 2-(4-morpholino)ethylamide⁽¹¹⁾ (190 mg, 0.78 mmol) there was obtained N²-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydro-
 5 quinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-[2-(4-morpholino)ethyl]amide (270 mg, 58%) as a white solid: MS (ESI) : 647 (M + H⁺) and 669(M + Na⁺).

(ii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-
 10 [2-(4-morpholino)ethyl]amide (260 mg, 0.4 mmol) there was obtained N²-[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine-N¹-[2-(4-morpholino)ethyl]amide (324 mg, 100%) : MS (ESI) : 591 (M + H⁺).

⁽¹¹⁾ L-tert-leucine 2-(4-morpholino)ethylamide was prepared by the reaction of L-tert-leucine
 15 with triphosgene to give 3-(S)-tert-butyloxazolidine-1,4-dione which was then treated with 4-(2-aminoethyl)morpholine.

Example 22

20 N²-[[4-(N-Hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N¹-(4-morpholine)amide



In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-
 25 butyldimethylsilyl)hydroxylamine (1.1 equivalents) was used instead of hydroxylamine hydrochloride, from N²-[[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-

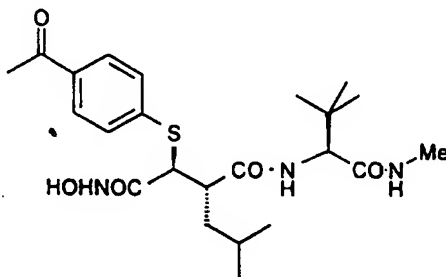
tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N¹-(4-morpholine)amide (415 mg, 0.76 mmol) there was obtained after treatment of the crude reaction mixture with HCl (2N, 0.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 15/85 to 50/50), N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N¹-(4-morpholine)amide (137 mg, 32%) as a white powder : m.p. 218-220°C ; ¹H-NMR (DMSO d-6) : 0.76 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 0.94 (m, 1H), 0.99 (s, 9H), 1.35-1.5 (m, 2H), 2.51-2.55 (m, H), 2.8-2.85 (m, 2H), 3.02 (m, 1H), 3.22 (s, 3H), 3.32-3.4 (m, 2H), 3.45 (d, 1H, J = 11.3 Hz), 3.55-3.75 (m, 6H), 4.82 (d, 1H, J = 8.8 Hz), 7.0 (d, 1H, J = 8.4 Hz), 7.2 (d, 1H, J = 1.8 Hz), 7.25 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz), 8.16 (d, 1H, J = 9.1 Hz), 8.95 (s br, 1H), 10.57 (s br, 1H) ; MS (ESI) : 563 (M + H⁺) and 585 (M + Na⁺).

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-butan-1,4-dioic acid 4-tert-butyl ester (375 mg, 0.89 mmol), described in Example 9, and N-(L-tert-leucine)-(4-morpholine)amide ⁽¹²⁾ (196 mg, 0.98 mmol) there was obtained N-[[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine]-N¹-(4-morpholine)amide (465 mg, 86%) as a white solid: MS (ESI) : 626 (M + Na⁺).

(ii) In a manner analogous to that described in Example 1 (iii), from N-[[2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine]-N¹-(4-morpholine)amide (460 mg, 0.76 mmol) there was obtained N-[[4-hydroxy-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]-N¹-(4-morpholine) (417 mg, 100%) : MS (ESI) : 548 (M + H⁺), 570 (M + Na⁺).

⁽¹²⁾ N-(L-tert-leucine)-(4-morpholine)amide was prepared by the reaction of L-tert-leucine with triphosgene to give 3-(S)-tert-butyloxazolidine-1,4-dione which was then treated with morpholine.

Example 23**N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide**

5

In a manner analogous to that described in the first paragraph of Example 1, except that O-(t-butyldimethylsilyl) hydroxylamine (1.2 equivalents) was used instead of hydroxylamine hydrochloride, from a mixture of N²-[4-hydroxy-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide and the corresponding 3R epimer

10 (400 mg, 0.89 mmol), there was obtained after treatment of the crude reaction mixture with HCl (2N, 0.5 ml) and purification by C18 preparative HPLC using as eluant a mixture of methanol and water/1% AcOH (gradient from 30/70 to 60/40), N²-[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (80 mg, 20%) as a white solid : m.p. 210-211°C; ¹H-NMR (DMSO d-6) : 0.79 (d, 3H, J = 6.6 Hz), 0.87 (d, 3H, J = 6.6 Hz), 0.9 (s, 9H), 1.04 (m, 1H), 1.39 (m, 1H), 1.49 (m, 1H), 2.56 (s, 3H), 2.57 (d, 3H, J = 3.7 Hz), 3.09 (m, 1H), 3.82 (d, 1H, J = 11.0 Hz), 4.2 (d, 1H, J = 9.2 Hz), 7.46 (d, 2H, J = 8.4 Hz), 7.8 (m, 1H), 7.85 (d, 2H, J = 8.4 Hz), 8.07 (d, 1H, J = 9.2 Hz), 9.07 (s br, 1H), 10.89 (s br, 1H) ; MS (ESI) : 488 (M + Na⁺),

and the other epimer, N²-[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide (183 mg, 44%) as a white solid : m.p. 220-221°C ; MS (ESI) : 466 (M + H⁺), 488 (M + Na⁺).

20

The starting material was prepared as follows:

(i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid 4-tert-butyl ester (5.0 g, 21.7 mmol) and di-[4-(2-methyl-1,3-dioxolan-2-yl)phenyl] disulfide⁽¹³⁾ (9.32 g), there was obtained a mixture of 2S-isobutyl-3S-[4-(2-methyl-1,3-

25

dioxolan-2-yl)phenyl]thiobutan-1,4-dioic acid 4-tert-butyl ester and the corresponding 3R epimer (5.53g, 60%) (3S:3R, 1:3) : MS (ESI): 447 (M + Na⁺).

This mixture was used unseparated for the next steps.

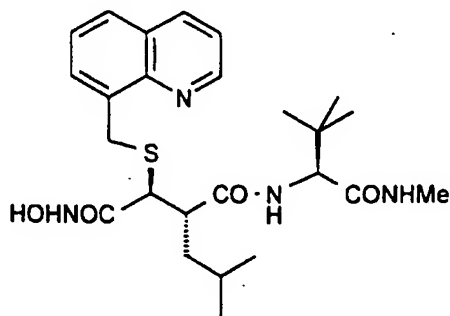
- 5 (ii) In a manner analogous to that described in Example 1 (ii), from the mixture of 2S-isobutyl-3S-[4-(2-methyl-1,3-dioxolan-2-yl)phenyl]thiobutan-1,4-dioic acid 4-tert-butyl ester and the corresponding 3R epimer (2.33 g, 5.49 mmol), there was obtained a mixture of N²-[2S-isobutyl-3S-{4-(2-methyl-1,3-dioxolan-2-yl)phenyl}thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide together with the corresponding 3R isomer (2.4 g, 79%) as an off
10 white solid: MS (ESI): 551 (M + H⁺), 573 (M + Na⁺), 589 (M + K⁺).

- (iii) Hydrochloric acid (2N, 800 µl) was added to a solution of the mixture of N²-[2S-isobutyl-3S-{4-(2-methyl-1,3-dioxolan-2-yl)phenyl}thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide together with the corresponding 3R isomer (1.74 g, 3.16 mmol) in acetone (17
15 ml) and the mixture was stirred at room temperature for 4 hours. The acetone was evaporated, the residue taken up in dichloromethane, washed with water and brine and dried over MgSO₄. The solvent was removed in vacuo to give a mixture of N²-[2S-isobutyl-3S-(4-acetylphenyl)-thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide together with the corresponding 3R isomer (1.52 g, 79%) as an off white solid: MS (ESI): 507 (M + H⁺), 529 (M + Na⁺).

20

- (iv) In a manner analogous to that described in Example 1 (iii), from mixture of N²-[2S-isobutyl-3S-(4-acetylphenyl)thio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide together with the corresponding 3R isomer (1.5 g, 2.96 mmol) there was obtained a mixture of N²-[4-hydroxy-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine-N¹-methylamide
25 and the corresponding 3R epimer (1.17 g, 88%) :MS (ESI): 451 (M + H⁺), 473 (M + Na⁺).

(13) Zeneca Ltd., (Bird, T. G. C.; Ple, P.), Patent EP0555068 (1993), in Example 7.

Example 24**N²-[4-(N-Hydroxyamino)-2S-isobutyl-3S-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N¹-methylamide**

5

To a solution of N²-[4-hydroxy-2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N¹-methylamide (460 mg) in DMF (5 ml) cooled at 0°C were added 1-hydroxybenzotriazole (270 mg), N-methylmorpholine (400 µl), O-(2,4-dimethoxybenzyl)hydroxylamine⁽¹²⁾ (366 mg) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (382 mg). The mixture was stirred at room temperature for 18 hours. The mixture was partitioned between water and ethyl acetate. The organic layer was washed with saturated sodium bicarbonate, brine, dried over MgSO₄ and filtered. The solvents were evaporated in vacuo to give crude N²-[4-(N-(2,4-dimethoxybenzyloxy)amino)-2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N¹-methylamide. This crude material was dissolved in

15 dichloromethane (10 ml). Trifluoroacetic acid (0.7 ml) was added. The mixture was stirred at room temperature for 15 minutes. The solvents were evaporated in vacuo. Methanol was added (20 ml) and the solids were filtered off. The filtrate was concentrated and purified by C18 preparative HPLC using as eluant a mixture of methanol and water/1%AcOH (gradient from 0/100 to 50/50) to give the title compound (76 mg): ¹H-NMR (DMSO d-6): 0.78 (d, 3H, J= 6.6 Hz), 0.86 (m, 12H), 1.04 (m, 1H), 1.5-1.3 (m, 2H), 2.56 (d, 3H, J= 4.4 Hz), 3.08 (m, 1H), 3.28 (d, 1H, J= 11 Hz), 4.20 (d, 1H, J= 9.2 Hz), 4.43 (d, 1H, J= 12.5 Hz), 4.54 (d, 1H, J= 12.5 Hz), 7.55 (m, 2H), 7.80 (m, 2H), 7.89 (m, 2H), 8.36 (m, 1H), 8.93 (m, 1H), 9.04 (s, 1H), 10.75 (s, 1H); MS (EI): 488 (M⁺).

20

25 ⁽¹²⁾ Prepared from 2,4-dimethoxybenzyl alcohol according to Grochowski E, Jurczak J; Synthesis, 1976, 682.

The starting material was prepared as follows:

- (i) In a manner analogous to that described in Example 1 (i), from 2R-isobutylbutan-1,4-dioic acid-4-tert-butyl ester (400 mg, 1.74 mmol) and di-(quinolin-8-yl)methyl disulfide⁽¹³⁾ (726 mg, 2.08 mmol), there was obtained 2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiobutan-1,4-dioic acid-4-tert-butyl ester (487 g, 1:1) contaminated with ca 20% of starting carboxylic acid.

MS (ESI): 404 (M + H⁺).

^(13a) Prepared from 8-mercaptomethylquinoline (Chem. Abstracts, 1961, 14458h)

- (ii) In a manner analogous to that described in Example 1 (ii), from 2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiobutan-1,4-dioic acid-4-tert-butyl ester (450 mg, 1.12 mmol), there was obtained N²-[2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (451 mg, 1:1).

- (iii) In a manner analogous to that described in Example 1 (iii), from N²-[2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthio-4-tert-butyloxysuccinyl]-L-tert-leucine-N¹-methylamide (450 mg) there was obtained N²-[4-hydroxy-2S-isobutyl-3(R/S)-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine-N¹-methylamide as the trifluoroacetate salt : MS (EI): 474 (M + H⁺).

Example 25

20

Typical tablet formulations for a compound of this invention or a pharmaceutically-acceptable salt thereof ('Compound X') are:

(a)	<u>Tablet Formulation 1</u>	<u>mg/tablet</u>
25	Compound X.....	100
	Lactose Ph.Eur.....	179
	Croscarmellose sodium.....	12
	Polyvinylpyrrolidone.....	6
	Magnesium stearate.....	3

30

5	(b)	<u>Tablet Formulation II</u>	<u>mg/tablet</u>
		Compound X.....	250
		Lactose Ph.Eur.....	215
		Croscarmellose sodium.....	20
		Polyvinylpyrrolidone.....	10
		Magnesium stearate.....	5

The tablets may be prepared by conventional procedures well known in the pharmaceutical art and may be film coated with typical coating materials such as hydroxypropylmethylcellulose.

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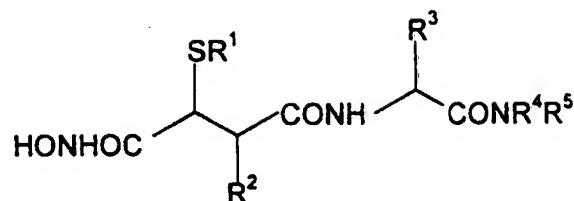
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CLAIMS

A compound of the formula (I):



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(I)

wherein:

- R¹ is aryl, arylC₁₋₆alkyl, heteroaryl or heteroarylC₁₋₆alkyl;
- 10 R² is hydrogen, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₆cycloalkyl, heteroaryl, heterocyclyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl, heterocyclylC₁₋₆alkyl or C₃₋₆cycloalkylC₁₋₆alkyl;
- R³ is C₁₋₆alkyl, C₂₋₆alkenyl, aryl, C₁₋₆alkyl, heteroarylC₁₋₆alkyl or the side-chain of a naturally occurring amino acid;
- 15 R⁴ is hydrogen, C₁₋₆alkyl, C₃₋₆cycloalkyl, C₄₋₆cycloalkenyl, arylC₁₋₆alkyl, heteroarylC₁₋₆alkyl or heterocyclylC₁₋₆alkyl;
- R⁵ is hydrogen or C₁₋₆alkyl; or R⁴ and R⁵ together with the nitrogen atom to which they are joined form a heterocyclic ring;

wherein any group or ring, in R¹-R⁵, is optionally substituted;

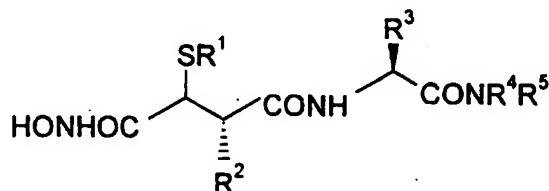
- 20 or a pharmaceutically acceptable salt or an in vivo hydrolysable ester thereof.

2. A compound according to claim 1 wherein R¹ is phenyl, phenylC₁₋₆alkyl, naphthylC₁₋₆alkyl, heteroaryl or heteroarylC₁₋₆alkyl wherein any of such rings is unsubstituted or substituted by one or two groups selected from halogen, C₁₋₆alkylcarbonyl, C₁₋₆alkylsulfonyl, trifluoromethyl, cyano, C₁₋₆alkyl, C₁₋₆alkoxy, cyanoC₁₋₆alkyl, or two adjacent carbon atoms on a phenyl ring are linked to form a methylenedioxy group.
- 25

3. A compound according to claim 1 wherein R¹ is phenyl and two adjacent carbon atoms are linked by -(CH₂)_m- wherein m is 3 or 4, by -NR^a-CO-(CH₂)_n- wherein R is hydrogen or C₁₋₆alkyl and n is 1 or 2, by -NR^a-COCH=CH-, -CO-NR^a-(CH₂)_n- or by -CONR^a-CH=CH-.

5

4. A compound according to claim 1 which is of the formula (II):



(II)

10 wherein R¹ is phenyl unsubstituted or substituted by one or two groups selected from halogen C₁₋₆alkylsulphonyl, trifluoromethyl, C₁₋₆alkyl, C₁₋₆alkoxy, cyano, C₁₋₆alkanoyl, cyanoC₁₋₆alkyl, or two adjacent carbon atoms on the phenyl ring are linked to form a methylenedioxy (-OCH₂O-) group; R² is isobutyl; R³ is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R⁴ is methyl, ethyl, n-propyl, isobutyl, tert-
15 butyldimethylaminoethyl or benzyl; and R⁵ is hydrogen or methyl, or R⁴ and R⁵ together with the nitrogen atom to which they are joined form a morpholino ring.

5. A compound according to claim 1 wherein R¹ is quinolinyl, isoquinolinyl, 1-methyl-2-oxodihydroquinolinyl, 1-methyl-2-oxotetrahydroquinolinyl, 2-methyl-1-oxodihydro-
20 isoquinolinyl or 2-methyl-1-oxotetrahydroisoquinolinyl; R² is isobutyl; R³ is isobutyl, tert-butyl, 1,1-dimethylmethylthiomethyl or benzyl; R⁴ is methyl, ethyl, n-propyl, isobutyl, tert-butyl, dimethylaminoethyl or benzyl; and R⁵ is hydrogen or methyl, or R⁴ and R⁵ together with the nitrogen atom to which they are joined form a morpholine ring.

25

6. A compound according to claim 1 which is:

- N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-phenylthiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3R-phenylthiosuccinyl]-L-tert-leucine- N^1 -methanamide;
5 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-fluorophenyl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,5-dichlorophenyl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine-
10 N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3R-(3,4-dimethoxyphenyl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
15 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-(methylsulfonyl)phenyl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3R-(4-cyanophenyl)thiosuccinyl]-L-tert-leucine- N^1 -
20 methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(3,5-di-(trifluoromethyl)phenyl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
25 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-benzylthiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(2-benzothiazol-2-yl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;
30 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3R-(6-hydroxynaphth-2-yl)thiosuccinyl]-L-tert-leucine- N^1 -methanamide;

N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(quinolin-2'-yl)thiosuccinyl]-L-tert-leucine- N^1 -methylamide;

N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-[3-(1-cyano-1-methylethyl)phenyl]thiosuccinyl]-L-tert-leucine- N^1 -methylamide;

5 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(2-chloro-4-fluorophenyl)thiosuccinyl]-L-tert-leucine- N^1 -methylamide;

N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(naphth-1-yl)thiosuccinyl]-L-tert-leucine- N^1 -methylamide;

N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-(1-cyano-1-methylethyl)phenyl)thiosuccinyl]-L-

10 tert-leucine- N^1 -methylamide;

N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine- N^1 -dimethylamide;

N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine- N^1 -(2-dimethylaminoethyl)amide;

15 N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine- N^1 -[2-(4-morpholino)ethyl]amide;

N^2 -[[4-(N-hydroxyamino)-2S-isobutyl-3S-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiosuccinyl]-L-tert-leucine]- N^1 -(4-morpholine)amide;

N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(4-acetylphenyl)thiosuccinyl]-L-tert-leucine- N^1 -

20 methylamide;

N^2 -[4-(N-hydroxyamino)-2S-isobutyl-3S-(quinolin-8-yl)methylthiosuccinyl]-L-tert-leucine- N^1 -methylamide;

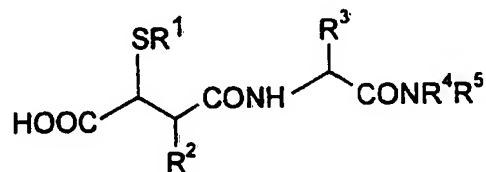
or a pharmaceutically acceptable salt thereof.

25 7. A pharmaceutical composition which comprises a compound according to any one of claims 1 to 6 and a pharmaceutically acceptable carrier.

8. The use of a compound according to any one of claims 1 to 6 for the manufacture of a medicament for treating disease conditions mediated by TNF.

9. A process for preparing a compound according to any one of claims 1-6 or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof which process comprises

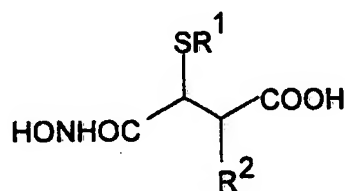
a) reacting a compound of the formula (III):



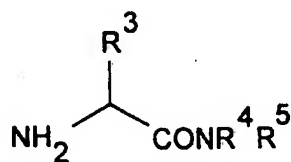
(III)

wherein R¹-R⁵ are as defined in claim 1, or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine or a salt thereof; or

10 b) coupling a compound of the formula (IV) with a compound of the formula (V):



(IV)



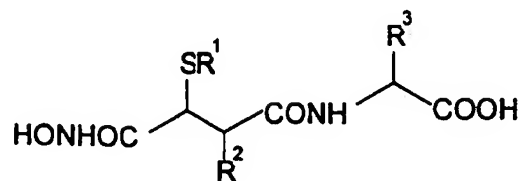
(V)

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wherein R¹-R⁵ are as defined in claim 1, under standard peptide coupling conditions; or

20

c) reacting a compound of the formula (VI) with compound of the formula (VII):



(VI)

5



(VII)

wherein R¹-R⁵ are as defined in claim 1,

10 wherein any functional group is protected, if necessary, and:

- i. removing any protecting groups;
- ii. optionally forming a pharmaceutically acceptable salt or in vivo hydrolysable ester.

10. A compound of the formula (III) as defined in claim 9.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/01164

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07C323/22 C07C323/60 C07C323/62 A61K31/16 A61K31/255

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 90 05719 A (BRITISH BIOTHEC PHARMACEUTICALS LIM., OXFORD, GB) 31 May 1990	1,2
Y	whole document, especially examples and claims	3-10
X	WO 95 09841 A (BRITISH BIOTHEC PHARMACEUTICALS LIM., OXFORD, GB) 13 April 1995	1,2
Y	whole document, especially examples and claims	3-10
X	WO 94 10990 A (BRITISH BIOTHEC PHARMACEUTICALS LIM., OXFORD, GB) 26 May 1994	1-10
	whole document, especially claim 13	
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

14 August 1997

Date of mailing of the international search report

10.09.97

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/01164

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	EP 0 236 872 A (F. HOFFMAN-LA ROCHE & CO. AG, BASLE, CH) 16 September 1987 claim 15, page 8, line 29 to page 10, line 14 -----	1-10

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